REGISTRATION REPORT

Part B

Section 5: Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB1310aH

Product name(s): Glosset Ace

Chemical active substances:

Aclonifen, 540 g/L
Flufenacet, 60 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem NV

Submission date: December 2021

Finalisation date: 25/08/2022

After commenting: 14/12/2022

Version history

When	What
December 2021	First submission of dossier by the applicant for approval of a new product
August 2022	First zRMS PL evaluation
December 2022	Corrections made by zRMS PL after commenting round

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Commodity/crop	Supported/ Not supported
Winter cereals	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of Aclonifen and Flufenacet in GLOB1310aH. The following analytical method for the determination of the active substances in the plant protection product GLOB1310aH has not previously been reviewed according to the Uniform Principles and is provided in support of this assessment.

Comments of zRMS:	The Validation of the Methods (Pomeroy, Dave, 2020. Study Number DNA5853, KCP 5.1.1) are acceptable for the determination of Aclonifen and Flufenacet in the product Glosset Ace (GLOB1310aH) to DG SANCO/3030/99 rev. 5. The LOQ and LOD for Aclonifen are set at the same level and defined as the lowest point on the linearity (0.002mg/mL, that equates to 2.0g/L). The LOQ and LOD for Flufenacet are set at the same level and defined as the lowest point on the linearity (0.002mg/mL, that equates to 2.0g/L). This submitted study has been validated in a proper manner.

Reference:	KCP 5.1.1
Report	Validation of the Methods of Determination of Aclonifen, Flufenacet and specified impurity in an SC Formulation containing Aclonifen and Flufenacet in Compliance with Good Laboratory Practice. Pomeroy, Dave, 2020. Study Number DNA5853
Guideline(s):	Yes (SANCO/3030/99 rev. 5)
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

The assay of Aclonifen and Flufenacet was performed using approximately 0.1 g of sample. The mass of the formulation was accurately recorded, transferred to a 100 mL volumetric flask and partially made to volume with Acetonitirile. The sample was sonicated for 5 minutes, allowed to cool to room temperature and made up to volume with Acetonitrile. The sample was then assayed by injecting each solution once into the HPLC-PDA under the following conditions:

HPLC-PDA Conditions – Aclonifen and Flufenacet validation:

Instrument:	Shimadzu HPLC-PDA
Mode:	Isocratic Reverse Phase
Column:	Agilent Zorbax Eclipse (75mm x 4.6mm)
Packing:	XDB-C8, 3.5µm
Eluent:	35% Acetonitrile
	65% Deionised Water at pH 3 adjusted with Formic Acid
Wavelength:	225nm
Injection Volume:	10µL
Flow Rate:	1.0mL/minute
Column temperature:	25°C
Data collection:	LabSolutions
Retention Times:	Aclonifen: approximately 31.9 to 32.3 minutes
	Flufenacet: approximately 29.2 to 29.4 minutes

LC-QQQ conditions – MS Spectral Analysis Aclonifen

Instrument:	Agilent LC-QQQ 6470 Mass Spectrometer		
Mode:	Isocratic Reverse Phase		
Column:	Agilent Zorbax Eclipse XDB-C8 (75mm x 4.6mm)		
Packing:	XDB-C8, 3.5µm		
Eluent:	50% Acetonitrile:50% Deionised water adjusted to pH 3 with Formic Acid		
Flow rate:	1mL/min		
Injection Volume:	10µL		
Column temperature:	25°C		
Data collection:	LabSolutions		
Retention Time:	Aclonifen: approximately 5.4 minutes		
Ionisation:	Positive	Sheath Gas Temperature:	250°C
Gas Temperature:	250°C	Sheath Gas Flow:	6L/min
Gas Flow:	6L/min	Capillary:	3500V
Nebulizer:	30psi	Nozzle voltage:	2000V

MRM Precursor ion (m/z)	MRM Precursor ion (m/z)	Dwell time (ms)	Fragmentor (V)	Collision energy (V)	Accelerator voltage (V)
265.0	248.0	200	121	20	5
265.0	182.0	200	121	32	5
265.0	127.1	200	121	52	5
265.0	77.1	200	121	52	5
265.0	51.2	200	121	83	5

Data acquisition: Mass Hunter

LC-QQQ conditions – MS Spectral Analysis Flufenacet

Instrument: Agilent LC-QQQ 6470 Mass Spectrometer

Mode:	Isocratic Reverse Phase		
Column:	Agilent Zorbax Eclipse XDB-C8 (75mm x 4.6mm)		
Packing:	XDB-C8, 3.5µm		
Eluent:	50% Acetonitrile:50% Deionised water adjusted to pH 3 with Formic Acid		
Flow rate:	1mL/min		
Injection Volume:	10µL		
Column temperature:	25°C		
Retention Time:	Flufenacet: approximately 5.1 minutes		
Ionisation:	Positive	Sheath Gas Temperature:	250°C
Gas Temperature:	250°C	Sheath Gas Flow:	6L/min
Gas Flow:	6L/min	Capillary:	3500V
Nebulizer:	30psi	Nozzle voltage:	2000V

MRM Precursor ion (m/z)	MRM Precursor ion (m/z)	Dwell time (ms)	Fragmentor (V)	Collision energy (V)	Accelerator voltage (V)
364.1	194.0	200	111	8	5
364.1	152.0	200	111	20	5
364.1	124.1	200	111	40	5
364.1	109.0	200	111	48	5
364.1	97.1	200	111	60	5

Data acquisition: Mass Hunter

Validation - Results and discussions

Full validation of the above-described method has been conducted in the same study.

Table 0-1: Methods suitable for the determination of active substances Aclonifen and Flufenacet in plant protection product GLOB1310aH

	Aclonifen	**Flufenacet**	**Acceptance criteria SANCO/3030/99 rev.5**
Author(s), year	Pomeroy, D. 2020		-
Principle of method	HPLC-PDA and LC-MS		-
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity was determined from sixteen injectonss of eight concentrations of standard (from blank to 1.0mg/mL). Declared Aclonifen content of 540 g/L, equates to a concentration of 0.54mg/mL which falls within the limits of the linearity range. The method is linear with a correlation coefficient R^2 of 0.9998.	The linearity was determined from sixteen injectonss of eight concentrations of standard (from blank to 0.1mg/mL). Declared Flufenacet content of 60g/L, equates to a concentration of 0.06mg/mL which falls within the limits of the linearity range. The method is linear with a correlation coefficient R^2 of 0.9999.	$R^2 \geq 0.99$
Precision – Repeatability Mean (%RSD)	Six samples of approximately 0.1g of sample were prepared in 100mL volumetric flasks.	Six samples of approximately 0.1g of formulation were prepared in 100mL volumetric	Aclonifen: %RSD less than 1.47 and Hr≤1 at 548.8g/L

	Aclonifen	Flufenacet	Acceptance criteria SANCO/3030/99 rev.5
	The samples were partially made to volume with Acetonitrile and sonicated for 5minutes. The samples were then injected into the HPLC-PDA. %RSD = 0.348 Hr = 0.237 n = 6	flasks. The samples were partially made to volume with Acetonitrile and sonicated for 5minutes. The samples were then injected into the HPLC-PDA. %RSD = 0.439 Hr = 0.215 n = 6	Flufenacet: %RSD less than 2.04 and Hr≤1 at 61.90g/L
Accuracy (% Recovery)	The sample contains 540g/L Aclonifen, this equates to 0.54mg/mL as the samples were made at 1.0mg/mL concentration. The samples were prepared for analysis by weighing the formulation blank at 1.0mg/mL and spiking at 0.54mg/mL using Aclonifen standard. At 540g/L: Mean Recovery = 101.6% %RSD = 0.362 Hr =0.247 n = 6	The sample contains 60g/L Aclonifen, this equates to 0.06mg/mL as the samples were made at 1.0mg/mL concentration. The samples were prepared for analysis by weighing 0.01g of the formulation blank and spike it with 0.6mL of 1.0mg/mL Flufenacet standard. At 60g/L: Mean Recovery = 100.8% %RSD = 0.422 Hr =0.207 n = 6	Aclonifen: Between 97%-103% %RSD less than 1.47 And Hr≤1 at 548.8g/L Flufenacet: Between 90%-110% %RSD less than 2.04 and Hr≤1 at 60.49g/L
LOQ Recovery	The LOQ and LOD are set at the same level and defined as the lowest point on the linearity (0.002mg/mL, that equates to 2.0g/L). At 2g/L: Mean Recovery = 101.8% %RSD = 2.280 Hr =0.669 n = 6	The LOQ and LOD are set at the same level and defined as the lowest point on the linearity (0.002mg/mL, that equates to 2.0g/L). At 2g/L: Mean Recovery = 98.51% %RSD = 3.397 Hr =0.993 n = 6	Aclonifen: Between 80%-120% %RSD less than 3.41 and Hr≤1 at 2.036g Flufenacet: Between 80%-120% %RSD less than 3.42 and Hr≤1 at 1.970g/
Interference/ Specificity	Aclonifen eluted at 9.6 minutes and there were no other peaks present at the same elution time as Aclonifen. In UV Spectral analysis the Aclonifen reference standard gave representative peak at 32.2 (195, 310, 395 nm and extinction after 420nm). The sample of the formulation gave	Flufenacet eluted at 29.2 minutes and there were no other peaks present at the same elution time as flufenacet. In UV Spectral analysis the Flufenacet reference standard gave representative peak at 29.2 with a spectral maxima below 190nm, a secondqry maxima at 240nM, reducing to	To confirm the species identity in the associated sprectra traces. To show no interference

	Aclonifen	Flufenacet	Acceptance criteria SANCO/3030/99 rev.5
	representative peak at 32.2 minutes (195, 310 and 395nm and extinction after 400nm), in a similar manner to the reference standard. This shows that the method is specific.	extinction by 280nm. The formulation sample gave a peak at 29.3 with a spectral maxima below 190nm and a secondary maxima at 240nm, reducing to extinction by 280nm, in a similar manner to the reference standards. This shows that the method is specific.	
Comment	-	-	-

Conclusion

The analytical method is suitable for the specific and accurate determination Aclonifen and Flufenacet in GLOB1310aH, with acceptable accuracy and precision. The validation parameters for the Aclonifen and Flufenacet methodologies have been met for this study under the SANCO/3030/99 rev.5 guidelines.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

During Annex I inclusion, a limit was set for the phenol content in Aclonifen technical. Although no phenol was detected in Aclonifen technical from Globachem and consequently no phenol will be present in the formulation, a method was developed to determine the concentration of phenol in GLOB1310aH.

Comments of zRMS:	The Validation of the Methods (Pomeroy, Dave, 2020. Study Number DNA5853, KCP 5.1.1) is suitable for the specific and accurate determination of the relevant impurity Phenol in GLOB1310aH, with acceptable accuracy and precision. The validation complies with the criteria of SANCO/3030/99 rev.5 guidelines. This submitted study has been validated in a proper manner.

Reference:	KCP 5.1.1
Report	Validation of the Methods of Determination of Aclonifen, Flufenacet and specified impurity in an SC Formulation containing Aclonifen and Flufenacet in Compliance with Good Laboratory Practice. Pomeroy, Dave, 2020. Study Number DNA5853
Guideline(s):	Yes (SANCO/3030/99 rev. 5)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The assay of phenol was performed using approximately 0.25g of sample and then transferred to a 25mL volumetric flask and partially made to volume with Acetonitrile. The sample was sonicated for 5 minutes, allowed to cool to room temperature, then made up to volume with acetonitrile. The sample was then assayed by injecting each solution once into the HPLC-DAD under the following conditions:

HPLC-PDA Conditions – Phenol:

Instrument:	Agilent HPLC-PDA
Mode:	Gradient Reverse Phase
Column:	Grace Alltima C8, (250mm x 4.6mm)
Packing:	C8, 5µm
Eluent:	A: Acetonitrile
	B: Deionised water at pH3 adjusted with formic acid
Wavelength:	270nm
Injection Volume:	20µL
Flow Rate:	1.0mL/minute
Column temperature:	25°C
Data collection:	LabSolutions
Retention Times:	approximately 5.9 to 6.0 minutes

HPLC-PDA Gradient conditions:

Time (minutes)	Eluent A Percentage	Eluent B Percentage
0.00	40	60
8.00	40	60
8.01	80	20
21.00	80	20
21.01	40	60
30.00	40	60

Between 8.00-8.01 minutes, the ratio of the eluent is changing from 40% to 80% for eluent A from 60% to 20% for eluent B. between 21.00-21.01 minutes, the ratio of eluent is changing from 80% to 40% for eluent A and from 20% to 60% for eluent B.

GC-MS Conditions – MS Spectral Analysis Phenol:

Instrument:	Shimadzu GC-MSD with HS-20 Headspace Sampler
Column:	DB-624 (30m x 1.4µm x 0.25mm)
Temperatures:	
Column:	40°C for 5 minutes, then 10°C/minute to 220°C held for 5 minutes
Injector:	33°C
Carrier gas:	Helium
Detector:	Scan 25-250m/z for MS Spectral Analysis
Data collection:	GCMS Solutions
Retention time:	Approximately 17.5minutes

Headspace conditions:

Cycle time:	28 minutes
Shake strength:	4/5

Oven temperature:	70°C
Loop Temperature:	150°C

Transfer Line Temperature: 160°C

A primary standard of reference grade phenol was prepared at a concentration of 1.0mg/mL. This was subsequently used to prepare the working standards with the following concentrations:
0.50mg/mL
0.25mg/mL
0.10mg/mL
0.05mg/mL
0.025mg/mL
0.01mg/mL
0.005mg/mL

Validation - Results and discussions

Table 0-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) GLOB1310aH

	Phenol	**Acceptance criteria SANCO/3030/99 rev.5**
Author(s), year	Pomeroy, D. 2020	-
Principle of method	HPLC- DAD	-
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearuty was determined from 16 injections of 8 concentrations ranging from a blank to 0.5mg/mL. R^2=0.9999	R^2=>0.99
Precision – Repeatability Mean n = 6 (%RSD)	To show the sample precision, 6 samples of approximately 0.25g of formulation sample. The samples were partially made to volume with Acetonitrile, sonicated for 5 minutes, allowed ti cool to room temperature before made up to volume with acetonitrile. The samples were injected into the HPLC-DAD. No detectable phenol above the LOQ Level of 0.5g/kg in the formulation, equating to 0.93g/Kg in the active ingledient as manufactured	Not aplicable
Accuracy n = 6 (% Recovery)	The recovery precision was performed by spiking phenol onto a formulation blank using certified reference material. Six separate solutions were prepared and then injected into the HPLC-DAD. Recovery 97.85% %RSD= 0.931	
Spectral analysis	UV: The phenol reference standard and the spiked formulation sample gave a peak at 6.0 minutes with a spectral maxima at 195nm, a secondary maxima at 215, a tertiary maxima at 270 and reducing to extinction by 290nm. MS: The phenol reference standar and the spiked sample agave a peak at 17.5 minutes ahowing the prumary ion at 94m/z with additional ions at 66 m/z and 39m/z. The UV and MS spectra for Phenol	To confirm the species identity in the associated spectra traces

	Phenol	Acceptance criteria SANCO/3030/99 rev.5
	confirmed the species identification	
Specificity	Phenol eluted at 6.0 minutes and there were no significant peaks present at the same elution time as phenol.	To show no interference
LOQ **Recovery at 0.5g/Kg**	The LOQ is defined as the lowest point on the linearity, which for phenol is 0.005mg/mL, this equates to 0.5g/kg as the samples were prepared at 10mg/mL concentration. The LOQ recovery was performed by spiking phenol onto the formulation blank. Six separate solution were prepared and injected into the HPLC-DAD. Mean Recovery=98.65% %RSD=3.272 Hr=0.776 N=6	Mean recovery between 75%-125% %RSD less than 4.22 Hr≤1 at 0.493g/kg
Comment	EFSA specification maximum 5.0g/kg in aclonifen	

Conclusion

The analytical method is suitable for the specific and accurate determination of the relevant impurity Phenol in GLOB1310aH, with acceptable accuracy and precision. The validation complies with the criteria of SANCO/3030/99 rev.5 guidelines.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Under current EU legislation, methods on formulants are not required. However, if a formulant is defined as relevant for toxicity (environment, health), then a method needs to be provided. There are however no formulants in GLOB1907bH that are defined as relevant for toxicity.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of Aclonifen or Flufenacet.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of aclonifen and flufenacet for the generation of pre-authorization data is given in the following tables.

Table 0-3: Validated methods for the generation of pre-authorization data - Aclonifen

Component of residue definition: Aclonifen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products,... (Residues)	Primary	0.01 mg/kg (wheat grain, barley grain, corn grain, tomato)	GC-ECD	EFSA, 2008
		0.02 mg/kg (lemon, sunflower seed)	GD-ECD	EFSA, 2008
		0.05 mg/kg (corn silage)	GC-ECD	EFSA, 2008
		0.01 mg/kg (potato)	GD-MSD	EFSA, 2008
		0.01 mg/kg (sunflower seed, dry pea, onion)	LC-MS/MS	EFSA, 2008
	Confirmatory (if required)	0.02 mg/kg (dry crops, high fat content crops, high water content crops) 0.01 mg/kg (dry crops)	GC/NPD GC/MSD	DAR (Germany), 2006
Animal products, food of animal origin,... (Residues)	Primary	Not relevant, no MRL proposed		
	Confirmatory (if required)	-	-	-
Soil (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.01 mg/kg (sandy clay loam, sandy silt loam) 0.01 mg/kg	GC-ECD GC-MSD	EFSA, 2008
	Confirmatory (if required)	-	-	-
Water (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.05 µg/L (tap water, mineral water, river water, pond water)	GC-ECD	EFSA, 2008
	Confirmatory (if required)	0.05 µg/L (river water, pond water)	GC-MSD	EFSA, 2008
Air (Exposure)	Primary	0.25 µg/m^3 (warm, humid air)	GC-ECD	EFSA, 2008

Component of residue definition: Aclonifen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	-	-	-
Body fluids and tissues (Toxicology)	Primary	Not relevant, because active substance is not classified as toxic or highly toxic (T/T+) This point will be addressed at the renewal of aclonfien.		
	Confirmatory (if required)			

The residue analytical methods for the determination of flufenacet in plants and animal matrices were evaluated in the DAR (France 1997) including its addenda. These data are out of protection and can be used in support of GLOB1310aH.

Table 0-4: Validated methods for the generation of pre-authorization data - Flufenacet

Component of residue definition: Flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expresses as flufenacet equivalents.				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Cereals, maize, sunflower and soybean (high water content, high protein/starch content (dry), high oil cntent)	Primary	0.05 mg/kg (straw: 0.1mg/kg)	GC-MS	SANCO/7469/VI/98 – 03 July 2003 EU agreed method according to Review of the existing MRLs for flufenacet (EFSA Journal 2012;10(4):2689)

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 0 can be applied.

The methods already submitted in accordance with the requirements set out in point 0 apply.

5.3.2 Description of analytical methods for the determination of residues Aclonifen (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Aclonifen:
Proposed uses: winter cereals
Residue definition: aclonifen for plants and foodstuffs of animal origin
MRL: 0.01 mg/kg for cereals, muscle fat liver, kidney, eggs and milk (reference MRL Reg (EU) 2021/1531)
Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 0-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Pplant, high water content	Aclonifen	0.01 mg/kg	Reg (EC) 2021/1531
Plant, high acid content		0.01 mg/kg	
Plant high protein/high starch content (dry commodities)		0.01 mg/kg	
Plant high oil content		0.01 mg/kg	
Plant difficult matrices (hops, spices, tea)		0.05 mg/kg	
Muscle	Aclonifen	0.01 mg/kg	
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Aclonifen	0.01 mg/kg	EFSA, 2008
Drinking water (Human toxicology)	Aclonifen	0.05 µg/L	EFSA, 2008
Surface water (Ecotoxicology)	Aclonifen	0.05 µg/L (river water, pond water) 0.04538 µg/L	EFSA, 2008 Lemna gibba EC50 Study 21 08 ALE 0001 (Juckeland, D., filed as KCP 9.5 in Part B9)
Air	Aclonifen	0.25 µg/m^3	EFSA, 2008
Tissue (meat or liver)	Aclonifen	Not required	Not classified as T / T+ EFSA, 2008
Body fluids		Not required	Not classified as T / T+ EFSA, 2008

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

Aclonifen:

An overview on the acceptable methods and possible data gaps for analysis of aclonifen in plant matrices is given in the following tables.

The analytical method for the analysis of Aclonifen in wheat (used in studies KCA 6.3-01 and KCA 6.3-02, dRR Section B7) has been validated by Eurofins Agroscience Services in Study N°S20-07421. This study is submitted as KCP 5.2-01 (Winter, O. and Graf, H., 2021) and the method validation part is summarised in Appendix 2.

Table 0-2: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: aclonifen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.01 mg/kg	Method DFG-S19 Determination by GC/ECD or GC/MSD	DE, 2006
	ILV	0.05 mg/kg	GC-ECD or GC/MSD	DE, 2006
	Confirmatory	0.01 mg/kg	GC/MSD	DE, 2006
High acid content	Primary	Not necessary, no intended use		
	ILV			
	Confirmatory			
High oil content	Primary	0.02 mg/kg	Method DFG-S19 Determination by GC/ECD or GC/MSD	DE, 2006
	ILV	Not necessary		
	Confirmatory (if required)	0.02 mg/kg 0.01 mg/kg	GC/NPD GC/MSD	DE, 2006
High protein/high starch content (dry)	Primary	0.01 mg/kg	Method DFG-S19 Determination by GC/ECD or GC/MSD	DE, 2006
	ILV	0.01 mg/kg	GC-ECD or GC/MSD	DE, 2006
	Confirmatory	0.01 mg/kg	GC-ECD or GC/MSD	DE, 2006
Difficult (if required, depends on intended use)	Primary	Not necessary		
	ILV			
	Confirmatory (if required)			

Table 0-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	EU Review of Aclonifen
Not required, because:	Grain: for the proposed uses the residues are expected to be < LOQ (<0.01mg/kg)

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

In the EFSA Scientific report (2008) a method for animal products was not necessary since no MRL was set. However, as described in EFSA (2016), an analytical method using HPLC-MS/MS was fully validated for enforcement of the proposed residue definition with an LOQ of 0.01 mg/kg in muscle, fat, liver, kidney, milk and eggs.

Table 0-2: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: aclonifen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary (DFG S19)	0.01 mg/kg	GC-ECD	DAR, 2006; EFSA, 2008
	ILV	Not available		
	Confirmatory (if required)	Not available		
Eggs	Primary (DFG S19)	0.05 mg/kg	GC-ECD	DAR, 2006; EFSA, 2008
	ILV	Not available		
	Confirmatory (if required)	Not available		
Muscle	Primary (DFG S19)	0.05 mg/kg	GC-ECD	DAR, 2006; EFSA, 2008
	ILV	Not available		
	Confirmatory (if required)	Not available		
Fat	Primary (DFG S19)	0.05 mg/kg	GC-ECD	DAR, 2006; EFSA, 2008
	ILV	Not available		
	Confirmatory (if required)	Not available		
Liver	Primary (DFG S19)	0.05 mg/kg	GC-ECD	DAR, 2006; EFSA, 2008
	ILV	Not available		
	Confirmatory (if required)	Not available		

Table 0-3: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Residues are <0.01 mg/kg in all animal matrices from the low dose group in the goat metabolism study (DAR, 2006) Since residues are below 0.01 mg/kg extraction efficiency is not needed.

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Aclonfien in soil is given in the following tables.

Table 0-4: Validated methods for soil

Component of residue definition: Aclonifen			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg (sandy clay loam, sandy silt loam) 0.01 mg/kg	GC-ECD GC-MSD	DAR, 2006; EFSA, 2008
Confirmatory	-	-	-

Comment (zRMS): According to Vol. 3, B.9.9.2.1, Addendum to the DAR, 12/2011, method that meets the requirements for soil is recommended. It should be amended during the renewal of the active substance Aclonifen.

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Aclonifen in surface and drinking water is given in the following tables.

Table 0-5: Validated methods for water (if appropriate)

Component of residue definition: Aclonifen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L (tap water, mineral water, river water, pond water)	GC-ECD	DAR, 2006; EFSA, 2008
	ILV	-	-	-
	Confirmatory	0.1 µg/L	GC-MSD	DE, 2006
Surface water	Primary	0.05 µg/L	GC/ESD	DAR, 2006; EFSA, 2008

Component of residue definition: Aclonifen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory	(river water, pond water)	GC/MSD	DAR, 2006; EFSA, 2008

An ILV method for drinking water will be addressed at the renewal of aclonifen.
Comment (zRMS): According to COMMISSION Regulation (EC) No 284/2013, an ILV for drinking water is required. It should be amended during the renewal of the active substance Aclonifen.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Aclonifen in air is given in the following tables.

Table 0-6: Validated methods for air (if appropriate)

Component of residue definition: Aclonifen			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.25 µg/m^3 (warm, humid air)	GC-ECD	DAR, 2006; EFSA, 2008
Confirmatory	-	-	-

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Not required as Aclonifen is not classified as toxic or very toxic.

This point should be amended at the renewal of the active substance Aclonifen.

Comment (zRMS): According to COMMISSION Regulation (EC) No 284/2013, an ILV for body fluids and tissues is required. It should be amended during the renewal of the active substance Aclonifen.

5.3.2.8 Other studies/ information

The analytical method for the analysis of Aclonifen in wheat grain (used in studies KCA 6.3-01 and KCA 6.3-02, dRR Section B7) has been validated by Eurofins Agroscience Services in Study N°S20-07421. This study is submitted as KCP 5.2-01 (Winter, O. and Graf, H., 2021) and the method validation part is summarised in Appendix 2.

In several ecotoxicological studies summarised in section B9 of the dRR (toxicity to aquatic organisms honeybees and non-target plants), analytical methods were used for the detection of the active substances Aclonifen and flufenacet in the different test mediums. The analytical part of these studies is summarised in Appendix 2.

5.3.3 Description of analytical methods for the determination of residues of flufenacet (KCP 5.2)

5.3.3.1 Overview of residue definitions and levels for which compliance is required

Flufenacet:

Proposed uses: winter cereals
Residue definition: sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent
MRL: 0.05 mg/kg for cereals (except barley and wheat), muscle, fat, kidney, offals, eggs; 0.1mg/kg barley, wheat, milk; 0.02mg/kg liver (Reg 1127/20174)
Compared to the residue definition proposed in the Draft Assessment Report - France, 1997 (incl. its addenda) the current legal residue definition is identical.

Table 0-9: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05 mg/kg (lowest MRL) 0.01 mg/kg (LOQ)	Reg. (EC) 1127/2014
Plant, high acid content		0.05 mg/kg (lowest MRL) 0.01 mg/kg (LOQ)	
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg (lowest MRL) 0.01 mg/kg (LOQ)	
Plant, high oil content		0.05 mg/kg (lowest MRL) 0.01 mg/kg (LOQ)	
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg (lowest MRL)	
Muscle	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05 mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Milk		0.01 mg/kg (lowest MRL) 0.01 mg/kg (LOQ)	
Eggs		0.05 mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Fat		0.05 mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Liver		0.02 mg/kg (lowest MRL) 0.02 mg/kg (LOQ)	
Kidney		0.05 mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Soil (Ecotoxicology)	Flufenacet	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Flufenacet	0.1 µg/L	General limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Flufenacet	0.005103 mg/L	Lemna gibba EC50 Study 21 08 ALE 0001 (Juckeland, D., filed as KCP 9.5 in Part B9)
Air	Flufenacet	LOQ=2.2µg/m^3	AOEL 0.017mg/kg bw/d
Tissue (meat or liver)		Not required	Not classified as T / T+
Body fluids			

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

Flufenacet:

All analytical methods are active substance data and were provided in the EU review of flufenacet and were considered adequate.

The analytical method for the analysis of flufenacet in wheat (used in studies KCA 6.3-01 and KCA 6.3-02, dRR Section B7) has been validated by Eurofins Agroscience Services in Study N° S20-09167. This study is submitted as KCP 5.2-02 (Winter, O. and Amann, S., 2021) and the method validation part is summarised in Appendix 2.

Table 0-10: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.05 mg/kg	GC-MS	Monograph Flufenacet, 1997
	ILV	0.05 mg/kg	GC-MS	
	Confirmatory	0.05 mg/kg	GC-MS	
High protein/starch content and dry	Primary	0.05 mg/kg 0.01mg/kg straw	GC-MS	
	ILV	0.05 mg/kg	GC-MS	
	Confirmatory	0.05 mg/kg 0.01mg/kg straw	GC-MS	
High oil	Primary	0.05 mg/kg	GC-MS	
	ILV	-		
	Confirmatory	-		
Difficult (if required, depends on intended use)	Primary	Not necessary		
	ILV			
	Confirmatory (if required)			

All analytical methods are active substance data and were provided in the EU review of flufenacet and were considered adequate. Therefore, for the proposed use on cereals reference can be made to the EU review.

5.3.3.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Flufenacet in animal matrices is given in the following tables.

Table 0-11: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents exemplified with analytical targets flufenacet, FOE 5043 oxalate (hydrate), FOE 5043 sulfonic acid (sodium salt), FOE 5043 thioglycolate sulfoxide determined as FOE 5043 trifluoroacetamide				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk Muscle Fat Kidney Liver	Primary	Milk: 0.01 mg/kg Bovine liver: 0.02 mg/kg Bovine kidney, muscle fat: 0.05 mg/kg	GC-MS	
	ILV	Liver: 0.05 mg/kg	GC-MS	
	Confirmatory (if required)	/	/	
Eggs	Primary	0.05 mg/kg	GC-MS	

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to 0.

Table 0-12: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Since residues ≥LOQ are not anticipated in commodities of animal origin data on extraction efficiency in products of animal origin are not reported in the present submission. EFSA, 2012: "*On the basis of the animal metabolism studies it is concluded that, after exposure to the maximum dietary burden (about 200 times lower than the dose level in the metabolism studies, [5 mg/kg bw/d]), residue levels in livestock commodities are expected to remain below the enforcement LOQ."*

5.3.3.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of flufenacet in soil is given in the following tables.

Table 0-13: Validated methods for soil

Component of residue definition: Flufenacet			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	HPLC-MS/MS	Monograph France, 1997
Confirmatory	/	/	/

For any special comments or remarkable points concerning the analytical methods for soil please refer to 0.

The primary method was evaluated during the Annex I Inclusion and was accepted by the European Commission as an adequate monitoring/enforcement method for the determination of flufenacet in soil. No confirmatory data were requested on that topic. However, this will be addressed at the renewal of flufenacet.

5.3.3.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of flufenacet in surface and drinking water is given in the following tables.

Table 0-14: Validated methods for water (if appropriate)

Component of residue definition: Flufenacet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.1 µg/L	HPLC-MS/MS	Monograph France, 1997
	Primary	0.10 µg/L	HPLC-MS/MS	Merdian, H., 2017
	ILV	0.10 µg/L	HPLC-MS/MS	Meseguer, C. 2018
Surface water	Primary	0.1 µg/L	HPLC-MS/MS	Monograph France, 1997
	Primary	0.10 µg/L	HPLC-MS/MS	Merdian, H., 2017
	ILV	0.10 µg/L	HPLC-MS/MS	Meseguer, C. 2018

The primary method was evaluated during the Annex I Inclusion and was accepted by the European Commission as an adequate monitoring/enforcement method for the determination of flufenacet in drinking and surface water. No confirmatory data were requested on that topic. However, a new primary method (Meridan H., 2017) and its corresponding ILV study (Meseguer, D., 2018) are submitted in the frame of this application.

For any special comments or remarkable points concerning the analytical methods for water please refer to 0.

5.3.3.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of flufenacet in air is given in the following tables.

Table 0-15: Validated methods for air (if appropriate)

Component of residue definition: Flufenacet			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	2.2 µg/m^3	HPLC-UV	Monograph France, 1997
Confirmatory	/	/	/

The primary analytical method was evaluated during the Annex I Inclusion and was accepted by the Eu-ro-pean Commission as an adequate monitoring/enforcement method for the determination of flufenacet in air. No confirmatory data were requested on that topic.

5.3.3.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Not required as flufenacet is not classified as toxic or very toxic.

The purpose of such analytical method for analysis of the active substance and relevant metabolites is the detection of intoxications in humans and animals. Relevant criteria for provision of a method as outlined in SANCO/825/00 rev. 8.1 are classification of the active substance or a relevant metabolite as toxic or very toxic, or classification according to GHS as follows: Acute toxicity (Cat. 1-3), CMR (Cat. 1) or STOT (Cat. 1). Flufenacet or any of its metabolites is not classified according to those categories.

No recommendation was provided during the EU peer review for analytes relevant for monitoring in body fluids and tissues. Therefore, reporting of a method for the determination of flufenacet in body fluids is not considered necessary. A fully validated method for the determination of flufenacet in body fluids and tissues should be provided at the renewal of the active substance to comply with the new regulation. En-forcement methods relevant to tissues are available and included in Table 5.3.3.3 However, a new method for determination of flufenacet in plasma is submitted in the frame of this dossier.

For the detailed evaluation of new studies it is referred to 0.

Table 0-16: Methods for body fluids and tissues (if appropriate)

Component of residue definition: flufenacet			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L	LC-MS/MS	Asekunowo, J. 2019

Comment (zRMS): According to COMMISSION Regulation (EC) No 284/2013, an ILV for body fluids and tissues is required. It should be amended during the renewal of the active substance Flufenacet.

5.3.3.8 Other studies/ information

The analytical method for the analysis of flufenacet in wheat (used in studies KCA 6.3-01 and KCA 6.3-02, dRR Section B7) has been validated by Eurofins Agroscience Services in Study N° S20-09167. This study is submitted as KCP 5.2-02 (Winter, O. and Amann, S., 2021) and the method validation part is summarised in Appendix 2.

In several ecotoxicological studies summarised in section B9 of the dRR (toxicity to aquatic organisms honeybees and non-target plants), analytical methods were used for the detection of the active substances Aclonifen and flufenacet in the different test mediums. The analytical part of these studies is summarised in Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.1	Pomeroy, D.	2020	Validation of the Methods of Determination of Aclonifen, Flufenacet and specified impurity in an SC Formulation containng Aclonifen and Flufenacet, in Compliance with Good Laboratory Practice DNA5853 Dvid Norris Analytical Laboratories Ltd, UK GLP Unpublished	N	Globachem NV
KCA 4.2	Winter, O. Graf, H.	2021	Validation of Analytical Methods for the Determination of Aclonifen in Different Matrices of Plant origin S20-07421 (GLC-2018V) Eurofins Agriscience Services, Germany GLP Unpublished	N	Globachem NV
KCA 4.2	Winter, O. Amann, S.	2021	Validation of Analytical Methods for the Determination of Flufenacet in Different Matrices of Plant origin S20-09167 (GLC-2019V) Eurofins Agriscience Services, Germany GLP Unpublished	N	Globachem NV
KCA 4.2	Merdian, H.	2016	Validation of the analytical method for the determination of flufenacet and its metabolites (M1, M2, M5, M7, M9, TFA and TFESA) in surface and drinking water. Study No.: S15-04126 Eurofins Agroscience Service Chem SAS, GLP	N	Task Force Flufenacet

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Unpublished		
KCA 4.2	Meseguer, C.	2018	Independent Laboratory Validation of the Analytical Method for the Determination of Flufenacet and its metabolites (M1, M2, M5, M7, M9 and TFESA) in surface and drinking water. S16-070-28 Eurofins Agroscience Services Chem SAS, Germany. GLP Unpublished	N	Task Force Flufenacet
KCA 4.2	Asekunowo, J.	2019	Development and Validation of an Analytical Method for the Determination of Flufenacet and its metabolite M9 in Plasma Study P 4840 G EAG Laboratories, Germany GLP Unpublished	N	Task Force Flufenacet
KCP 5.2 (filed as KCP 10.2.1)	Juckeland, D.	2021a	Acute toxicity of GLOB1310aH to *Daphnia magna* in a 48-hour static test Verification of the concentration of Aclonifen and Flufenacet in the test solutions Study 21 48 ADL 0001 (21 35 CRA 0003) Biochemagrar, Germany GLP Unpublished	N	Globachem NV
KCP 5.2 (filed as KCP 10.2.1)	Juckeland, D.	2021b	Effects of GLOB1310aH on *Lemna gibba* in a growth inhibition test under semi-static test conditions. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Study 21 48 ADL 0001 (21 35 CRA 0001) Biochemagrar, Germany GLP Unpublished	N	Globachem NV
KCP 5.2 (filed as KCP 10.2.1)	Juckeland, D.	2021c	Effects of GLOB1310aH on *Myriophyllum spicatum* in a semi-static water-sediment system. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Study 21 48 AMS 0001 (21 35 CRA 0004) Biochemagrar, Germany GLP	N	Globachem NV

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Unpublished		
KCP 5.2 (filed as KCP 10.2.1)	Juckeland, D.	2021d	Effects of GLOB1310aH on *Desmodesmus subspicatus* in an algal growth inhibiton test. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Study 21 48 AAL 0017 Biochemagrar, Germany GLP Unpublished	N	Globachem NV
KCP 5.2 (filed as KCP 10.2.1)	Juckeland, D.	2021e	Effects of GLOB1310aH on *Pseudokirchneriella subcapitata* in an algal growth inhibiton test. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Study 21 48 AAL 0001 Biochemagrar, Germany GLP Unpublished	N	Globachem NV
KCP 5.2 (submitted as KCP 10.3.1.1)	Amsel, K.	2021	Acute toxicity of GLOB1310aH to the bumblebee *Bombus terrestris* L. under laboratory conditions Determination of the concentration of aclonifen and flufenacet in feeding solutions and test solutions by LC-MS/MS Study No. 21 48 BBA 0001 (~~21 35 CRA 0006~~ 21 35 CRB 0006) Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 5.2 (submitted as KCP 10.3.1.2)	Dreßler, K.	2021	Chronic toxicity of GLOB1310aH to the honey bee *Apis mellifera* L. under laboratory conditions. Determination of the concentrations of aclonifen and flufenacet in feeding solutions by LC-MS/MS Study No. 21 48 BAC 0004 (21 35 CRB 0004) Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 5.2 (submitted	Hänsel, M.	2021	GLOB1310aH – Repetaed exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions. Determination of the concentrations of aclonifen and flufenacet in final diets by LC-MS/MS	N	Globachem NV

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
as KCP 10.3.1.2)			Study No. 21 48 BLC 0003 (21 35 CRB 0005) Biochem Agrar GmbH, Germany GLP Unpublished		
KCP 5.2 (Submitted as KCP 10.6.2)	Friedemann, A.	2021	Effects of GLOB1310aH on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions. Study No. 21 46 PSE 0001 (21 35 CRP 0002) Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 5.2 (Submitted as KCP 10.6.2)	Friedemann, A.	2021	Effects of GLOB1310aH on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions Verification of the concentration of Aclonifen and Flufenacet in the spray solution specimens Study No. 21 46 PVV 0001 (21 35 CRP 0001) Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
-	-	-	-	-	-

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	**Owner**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	**Owner**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Aclonifen

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The method Validation of Analytical Methods for the Determination of Aclonifen in Different Matrices of Plant Origin. Winter, O., Graf, H., 2021. Report S20-07421 (GLC-2018V) was successfully validated for the determination aclonifen in wheat (green plant, grain and straw) from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg according to the guidance documents SANCO/825/00, rev. 8.1 and SANCO/3029/99, rev. 4. After the experimental end of this study the new guideline SANTE/2020/12830, Rev.1, which supersedes guidelines SANCO/825/00, rev. 8.1 and SANCO/3029/99 rev. 4, was published. The data obtained within this validation study are mostly compatible with the new guideline. This submitted study has been validated in a proper manner.

Reference:	KCA 4.2
Report	Validation of Analytical Methods for the Determination of Aclonifen in Different Matrices of Plant Origin. Winter, O., Graf, H., 2021. Report S20-07421 (GLC-2018V). Eurofins Agroscience Services, Germany
Guideline(s):	Yes, SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Study Objective
The objective of the study was to validate an analytical method for the determination of aclonifen in wheat (green plant, grain and straw) according to the guidance documents SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1 of the European Commission with an intended limit of quantification (LOQ) of

0.01 mg/kg.

After the experimental end of this study the new guideline SANTE/2020/12830 Rev. 1; which supersedes guidelines SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 was published. The data obtained within this validation study are mostly compatible with the new guideline.

Materials and methods and Results

Analytical Procedure
In brief, samples of wheat (green plant, grain and straw) were extracted with acetonitrile/water (4/1, v/v). The extracts were cleaned by centrifugation and filtration.

Selectivity
Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions per analyte were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.

Matrix Effects
Matrix effects on the detection of aclonifen in extracts of wheat (green plant, grain and straw) were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at eight (8) concentration levels ranging from 0.100 ng/mL to 6.00 ng/mL. This range corresponds to 0.002 mg/kg to 0.12 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract.
The calibration curves obtained for both mass transitions of all analytes and all matrices were linear since coefficients of determination (R^2) were ≥ 0.98. Linear regression was performed with 1/x-weighting.

Quantification
Quantification was performed by using linear regression. The injections of the calibration solutions were spread evenly over the whole analytical sequence.

Accuracy and Precision
Accuracy was determined by fortification of control samples with known amounts of the test / reference items and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). The analytes were fortified jointly and quantified separately. The following recoveries were obtained:

Aclonifen							
Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Mass Transition *m/z* 265→248 (Quantification)							
Wheat (green plant)	0.01	93, 88, 93, 92, 84	90	4.4	5	88	5.0
	0.1	83, 83, 84, 85, 92	85	4.4	5		
Wheat (grain)	0.01	91, 87, 89, 84, 89	88	3.0	5	89	2.9
	0.1	87, 92, 92, 87, 90	90	2.8	5		
Wheat (straw)	0.01	72, 75, 73, 78, 73	74	3.2	5	74	2.7
	0.1	72, 75, 72, 76, 74	74	2.4	5		
Mass Transition *m/z* 265→182 (Confirmation)							
Wheat (green plant)	0.01	95, 101, 103, 89, 98	97	5.7	5	91	8.4
	0.1	83, 82, 86, 84, 91	85	4.2	5		
Wheat (grain)	0.01	91, 90, 95, 85, 90	90	4.0	5	89	3.8
	0.1	85, 90, 90, 84, 89	88	3.3	5		
Wheat (straw)	0.01	83, 73, 74, 73, 78	76	5.7	5	75	4.2
	0.1	72, 76, 74, 75, 76	75	2.2	5		

All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg for two (2) mass transitions are within 70 – 110 % with relative standard deviations ≤ 20 % and thereby comply with the standard acceptance criteria of the guidance documents SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The LOQ is the lowest validated fortification level for each analyte and was thus successfully established at 0.01 mg/kg in wheat (green plant, grain and straw) for the two (2) mass transitions. The LOD was set at 0.003 mg/kg for all analytes and matrices, which is 30 % of the LOQ.

Stability of Analytes in Stock and Fortification Solutions
Aclonifen was found to be stable for 258 days when prepared in acetonitrile and stored at typically 1 °C to 10 °C in the dark.

Stability of Analytes in Solvent Calibration Solutions
Aclonifen was found to be stable for 22 days when prepared in acetonitrile / water (4+1, v+v) and stored at typically 1 °C to 10 °C in the dark.

Stability of Analytes in Sample Extracts
Aclonifen was found to be stable in final extracts of all matrices at least 22 days when stored at typically 1 °C to 10 °C in the dark.

Conclusions
The method was found to be valid according to the guidance documents SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1 for the determination of aclonifen in/on wheat (green plant, grain and straw) with the tested LOQ of 0.01 mg/kg.

A 2.1.2.1.1.2 Independent laboratory validation

Not required

A 2.1.2.1.1.3 Confirmatory method

No confirmatory method is required

A 2.1.2.1.1.4 Extraction efficiency

No new or additional studies have been submitted

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.7 Other Studies/ Information

In several residue studies and ecotoxicological studies summarised in sections B7 and B9 of the dRR, analytical methods were used for the detection of the active substances Aclonifen and flufenacet in the different test mediums. The analytical part of these studies is summarised here.

Comments of zRMS:	The method Validation of Analytical Methods for the Determination of Aclonifen in Different Matrices of Plant Origin. Winter, O., Graf, H., 2021. Report S20-07421 (GLC-2018V) was successfully validated for the determination aclonifen in wheat (green plant, grain and straw) from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg according to the guidance documents SANCO/825/00, rev. 8.1 and SANCO/3029/99, rev. 4. After the experimental end of this study the new guideline SANTE/2020/12830, Rev.1, which supersedes guidelines SANCO/825/00, rev. 8.1 and SANCO/3029/99 rev. 4, was published. The data obtained within this validation study are mostly compatible with the new guideline.

	This submitted study has been validated in a proper manner.

Reference: KCP 5.2

Report: Validation of Analytical Methods for the Determination of Aclonifen in Different Matrices of Plant Origin. Winter, O., Graf, H., 2021. Report S20-07421 (GLC-2018V). Eurofins Agroscience Services, Germany (filed in section B7)

Guideline(s): Yes, SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and Methods

Method reference: Method 00950/M002 as published in Document MCA, Section 4(1)
Extraction: extraction with acetonitrile/water (4/1 v/v)
Detection: Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
Intended LOQ: 0.01 mg/kg
Intended LOD: 30% of the LOQ

Chromatographic conditions:
-HPLC system: 1290 Infinity Bynary LC System, Agilent Technologies (UHPLC, ≤1200bar)
-Column: Ascentris Express C18 (50mm x 2.1mm, 2.7µm)
-Column temperature: 60°C
-Injection volume: 5µL
-Mobile phases: Eluent A: Acetonitrile + 0.2% formic acid
Eluent B/ Water + 0.2% formic acid
-Gradient:

Time (min)	%Eluent A	%Eluent B	Flow (µL/min)
0.00	30	70	700
5.00	85	15	700
5.10	30	70	700
6.25	30	70	700

-Retention time: 2.1min

Mass spectrometric conditions:
MS system: TripleQuad 6500 System, SCIEX
Ionisation type: Electrospray ionisation
Polarity: Positive ion mode
Scan type: MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage: 5500V
Ionspray turboheader: 600°C
Curtain gas (CUR): Nitrogen set at 25 Gas flow 1: Nitrogen set at 40
Collision gas (CAD): Nitrogen set as 6 Gas flow 2: Nitrogen set at 60

Analyte monitored	Mass transition monitored (m/z)	Declustering potential (DP) (V)	Entrance Potential (V)	Collision Energy (eV)	Cell exit potential (V)	Dwell time (ms)
Aclonifen	265→248	75	10	25	15	100
	265→182	75	10	41	15	100
	267→250	75	10	25	15	100

Quantification
Quantification was performed using a calibration curve that fulfilled the above given criteria. The injection of standard solutions was spread evenly over the whole analytical sequence. The linear regression equation was used for calculation of the analyte concentrations.

Results

Selectivity
LC-MS/MS determination was conducted by monitoring two (2) mass transitions (m/z 265→248 and m/z 265→182). Due to enhanced sensitivity mass transition 265→248 m/z is proposed to be used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation. A reagent blank and two (2) control samples per matrix were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analyte. For both mass transitions, the samples showed no significant interference above 30 % of LOQ at the retention time of the analyte in any investigated matrix, therefore showing that the method is highly specific. Blank correction was not performed

Matrix effects
The effect of wheat (green plant, grain and straw) on the LC-MS/MS response was assessed by comparing peak areas of matrix-matched standards (90 % matrix amount) with solvent standards at identical nominal concentrations. Matrix suppression or enhancement was < 20 % for all investigated matrices and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at eight (8) concentration levels ranging from 0.100 ng/mL to 6.00 ng/mL. This range corresponds to a fortification level of 0.002 mg/kg to 0.12 mg/kg and thus covers the range from no more than 30 % of the limit of quantification (LOQ) and at least + 20 % of the highest analyte concentration detected in a (diluted) sample extract. The calibration curves obtained for both ion mass transitions and all matrices were linear since coefficients of determination (R^2) were ≥ 0.980.
Accuracy and precision
Accuracy was determined by fortification of control samples with known amounts of the test / reference item and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). Five (5) recovery determinations were performed at 0.01 mg/kg and at 0.1 mg/kg, respectively. Analysis was performed by single extraction and single injection. All mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 and SANCO/3029/99, rev. 4, since mean recoveries were in the range of 70 - 110 % relative standard deviations of ≤20 % for aclonifen in wheat (green plant, grain and straw) at each level.

LOQ and LOD
The LOQ of the method is defined as the lowest analyte concentration at which the methodology had been successfully validated. Thus, an LOQ of 0.01 mg/kg was confirmed for aclonifen in wheat.

Stability of Stock and Fortification Solutions
The stock solution prepared in acetonitrile were stored at typically 1 °C to 10 °C for 258 days in the dark, which was sufficient to cover the length of time they were used in this study. After this time freshly prepared dilutions of the stock solution were compared to freshly prepared dilutions of a freshly prepared stock solution by triplicate injection. One (1) mass transition was evaluated. The mean peak area of the stored diluted stock solution was within ± 20 % of the mean peak area of the freshly prepared diluted stock solution indicating that stock solutions are stable when stored at 1 °C to 10 °C in the dark for at least 258 days. Fortification solutions were prepared in the same solvent as the stock solutions and were also stored at typically 1 °C to 10 °C in the dark. Therefore, investigation of the stability of fortification

solutions was not necessary.

Stability of Solvent Calibration Solutions
The calibration solutions prepared in acetonitrile / water (4+1, v+v) were stored at typically 1 °C to 10 °C for 22 days in the dark, which was sufficient to cover the length of time they were used in this study. After this time calibration solutions were compared by single injection to a freshly prepared solvent calibration. The peak areas of the stored solvent calibration solutions were within ± 20 % of the freshly prepared calibration indicating that solvent standards solutions are stable when stored at 1 °C to 10 °C in the dark for at least 22 days.

Stability of Analytes in Sample extracts
Following the first analysis, the final extracts of fortified samples together with two (2) control sample extract were stored at typically 1 °C to 10 °C in the dark for 21 - 24 days. After this period, the final extracts were re-analysed against freshly prepared calibration standards. One (1) mass transition was evaluated. The mean recovery value(s) of the re-analysed extracts were in the range of 70 - 120 % and within ± 20 % of the original result. Therefore, extracts are considered to be stable when stored at 1 °C to 10 °C for at least 21 days in the dark.

Conclusions

The method was successfully validated for the determination aclonifen in wheat (green plant, grain and straw) from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg according to the guidance documents SANCO/825/00, rev. 8.1 and SANCO/3029/99, rev. 4.
After the experimental end of this study the new guideline SANTE/2020/12830, Rev.1, which supersedes guidelines SANCO/825/00, rev. 8.1 and SANCO/3029/99 rev. 4, was published. The data obtained within this validation study are mostly compatible with the new guideline.

Comments of zRMS:	The analytical phase of the study 21 48 ADL 0003 (21 35 CRA 0003) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 256.0 µg/L for Aclonifen and 0.4282 µg/L for Flufenacet. LOD was 28.98 µg/L for Aclonifen and 0.4282 µg/L for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Acute toxicity of GLOB1310aH to Daphnia magna in a 48-hour static test Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Juckeland, D., 2021a. Report 21 48 ADL 0003 (21 35 CRA 0003). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania. All reported concentrations are pure active ingredient, i.e. they were corrected for purity.

An external matrix-matched calibration with the analytical reference items including 8 calibration levels was performed.

Calibration data Aclonifen
Błąd! Nie można odnaleźć źródła odwołania.

Calibration data Flufenacet

Błąd! Nie można odnaleźć źródła odwołania.*Method validation data*
Błąd! Nie można odnaleźć źródła odwołania.

Method validation results Aclonifen

Validation level	Nominal concentration [µg/L]	Analysed concentration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	nd nd	-	-	-
Low (LOQ)	256.0	237.7 243.2 236.2 245.1 245.5	241.6	94.3	1.8
High	7357	7860 8115 7314 7118 7158	7602	102.1	5.9

nd – Aclonifen was not detected; LOQ = 256.0 µg/L; LOD = 3.775 µg/L

Method validation results Flufenacet

Validation level	Nominal concentration [µg/L]	Analysed concentration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	-0.06935 -0.06968	-	< LOD < LOD	-
Low (LOQ)	28.98	27.86 28.26 28.95 28.32 27.94	28.26	97.5	1.5
High	832.8	901.0 913.7 859.1 839.1 875.1	878.2	105.4	3.5

LOQ =28.98 µg/L; LOD = 0.4282 µg/L

- **Błąd! Nie można odnaleźć źródła odwołania.**

Analysis results of Aclonifen in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		48 h spent	
1	0.000	40	nd	-	nd	-

2	1056	40	1092	103.4	1094	103.7
3	1689	40	2016	119.3	1854	109.7
4	2698	62.5	2902	107.5	3061	113.4
5	4329	100	5092	117.6	4879	112.7
6	6921	133.3	7397	106.9	7889	114.0
7	11062	200	11762	106.3	10580	95.6

nd – Aclonifen was not detected; LOQ = 256.0 µg/L; LOD = 3.775 µg/L

Analysis results of Flufenacet in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		48 h spent	
1	0.000	40	< LOD	-	< LOD	-
2	119.5	40	131.6	110.1	130.5	109.2
3	191.2	40	219.2	114.6	215.9	112.9
4	305.4	62.5	357.2	116.9	331.2	108.4
5	490.0	100	529.9	108.1	584.6	119.3
6	783.5	133.3	866.3	110.6	876.0	111.8
7	1252	200	1307	104.4	1323	105.6

LOQ = 28.98 µg/L; LOD = 0.4282 µg/L

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 48 ALE 0001 (21 35 CRA 0001) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 0.04508 µg/L for Aclonifen and 0.005103 µg/L for Flufenacet. LOD was 0.01328 µg/L for Aclonifen and 0.001506 µg/L for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Effects of GLOB1310aH on *Lemna gibba* in a growth inhibition test under semi-static test conditions. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Juckeland, D., 2021b. Report 21 48 ALE 0001 (21 35 CRA 0001). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania. All reported concentrations are pure active ingredients, i.e. they were corrected for purity.

An external matrix-matched calibration with the analytical reference items including 8 calibration levels was performed.

Calibration data Aclonifen

Błąd! Nie można odnaleźć źródła odwołania.

Calibration data Flufenacet

Błąd! Nie można odnaleźć źródła odwołania.*Method validation data*
Błąd! Nie można odnaleźć źródła odwołania.

Method validation results Aclonifen

Validation level	Nominal concentration [µg/L]	Analysed con-centration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	nd nd	-	-	-
Low (LOQ)	0.04508	0.03789 0.03588 0.03804 0.03901 0.04166	0.03849	85.4	5.5
High	28.90	25.50 26.74 28.51 27.03 27.37	27.03	93.5	4.0

nd – Aclonifen was not detected; LOQ = 0.04508 µg/L; LOD = 0.01328 µg/L

Method validation results Flufenacet

Validation level	Nominal concentration [µg/L]	Analysed con-centration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	< LOD < LOD	-	-	-
Low (LOQ)	0.005103	0.004520 0.004669 0.004589 0.004606 0.004674	0.004611	90.4	1.4
High	3.271	3.330 3.253 3.363 3.245 3.257	3.290	100.6	1.6

LOQ = 0.005103 µg/L; LOD = 0.001506 µg/L

- **Błąd! Nie można odnaleźć źródła odwołania.**
-

Analysis results of Aclonifen in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		2 days spent	
1	0.000	2	nd	-	nd	-
2	0.1824	2	0.09415	51.6	0.1498	82.1
3	0.5463	2.667	0.3736	68.4	0.4444	81.4
4	1.640	8	1.337	81.5	1.201	73.2
5	4.919	10	3.544	72.0	3.737	76.0

6	14.76	28.57	11.06	75.0	9.781	66.3
7	44.27	80	40.20	90.8	42.74	96.5
			2 days fresh		4 days spent	
1	0.000	2	< LOD	-	< LOD	-
2	0.1824	2	0.1559	85.5	0.1526	83.7
3	0.5463	2.667	0.4637	84.9	0.5301	97.0
4	1.640	8	1.407	85.8	1.370	83.5
5	4.919	10	3.955	80.4	4.004	81.4
6	14.76	28.57	11.81	80.0	11.86	80.4
7	44.27	80	36.28	82.0	41.46	93.6
			4 days fresh		7 days spent	
1	0.000	2	< LOD	-	< LOD	-
2	0.1824	2	0.1546	84.8	0.1193	65.4
3	0.5463	2.667	0.7086	129.7	0.3888	71.2
4	1.640	8	1.321	80.5	1.234	75.2
5	4.919	10	4.094	83.2	3.638	74.0
6	14.76	28.57	16.23	110.0	11.16	75.6
7	44.27	80	42.02	94.9	40.21	90.8

nd – Aclonifen was not detected; LOQ = 0.04508 µg/L; LOD = 0.01328 µg/L

Analysis results of Flufenacet in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		2 days spent	
1	0.000	2	nd	-	< LOD	-
2	0.02065	2	0.02042	98.9	0.02293	111.0
3	0.06184	2.667	0.07286	117.8	0.07321	118.4
4	0.1856	8	0.2735	147.3	0.2003	107.9
5	0.5569	10	0.5817	104.5	0.5694	102.3
6	1.670	28.57	1.689	101.1	1.693	101.3
7	5.011	80	5.587	111.5	5.349	106.7
			2 days fresh		4 days spent	
1	0.000	2	< LOD	-	< LOD	-
2	0.02065	2	0.02625	127.1	0.02156	104.4
3	0.06184	2.667	0.06624	107.1	0.06873	111.1
4	0.1856	8	0.1967	106.0	0.2044	110.1
5	0.5569	10	0.6311	113.3	0.6025	108.2
6	1.670	28.57	1.780	106.6	1.782	106.7
7	5.011	80	5.430	108.4	5.463	109.0
			4 days fresh		7 days spent	
1	0.000	2	< LOD	-	< LOD	-
2	0.02065	2	0.02394	115.9	0.02033	98.5
3	0.06184	2.667	0.06157	99.6	0.06246	101.0
4	0.1856	8	0.1955	105.3	0.1857	100.1
5	0.5569	10	0.5496	98.7	0.5825	104.6
6	1.670	28.57	1.728	103.5	1.656	99.2
7	5.011	80	5.106	101.9	5.322	106.2

nd – Flufenacet was not detected; LOQ = 0.005103 µg/L; LOD = 0.001506 µg/L

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 48 AMS 0001 (21 35 CRA 0004) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 0.8052 µg/L for Aclonifen and 0.09116 µg/L for Flufenacet. LOD was 0.2346 µg/L for Aclonifen and 0.02635 µg/L for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Effects of GLOB1310aH on *Myriophyllum spicatum* in a semi-static water-sediment system. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Juckeland, D., 2021c. Report 21 48 AMS 0001 (21 35 CRA 0004). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes

Acceptability: Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania. All reported concentrations are pure active ingredients, i.e. they were corrected for purity.

An external matrix-matched calibration with the analytical reference items including 8 calibration levels was performed.

Calibration data Aclonifen
Błąd! Nie można odnaleźć źródła odwołania.

Calibration data Flufenacet

Błąd! Nie można odnaleźć źródła odwołania.*Method validation data*
Błąd! Nie można odnaleźć źródła odwołania.*Method validation results Aclonifen for overlaying water*

Validation level	Nominal concentration [µg/L]	Analysed concentration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	nd nd	-	-	-
Low (LOQ)	0.8052	0.8178 0.8180 0.8524 0.7656 0.7954	0.8098	100.6	4.0
High	728.1	769.4 748.3 787.2 741.1 773.7	764.0	104.9	2.5

nd – Aclonifen was not detected; LOQ = 0.8052 µg/L; LOD = 0.2346 µg/L (regarding $DF_{analytical}$ = 1)

Method validation results Flufenacet for overlaying water

Validation level	Nominal concentration [µg/L]	Analysed concentration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	0.001232 0.001165	-	< LOD < LOD	-
Low (LOQ)	0.09116	0.09373 0.09005 0.09027 0.08797 0.09049	0.09050	99.3	2.3
High	82.42	86.47 85.04 85.24 84.35 84.30	85.08	103.2	1.0

LOQ = 0.09116 µg/L; LOD = 0.02635 µg/L (regarding $DF_{analytical}$ = 1)

Concentrations of the target analytes in the blanks were < of its respective method LOD (i.e. 0.2346 µg/L for Aclonifen and 0.02635 µg/L Flufenacet for overlaying water; 0.2337 µg/L for Aclonifen and 0.02635 µg/L Flufenacet for pore water; 25.51 mg/kg for Aclonifen and 2.877 mg/kg Flufenacet for sediment; (regarding DFanalytical)).

The mean recovery values ranged from 99.3 to 104.9% (as shown in Table 14 and 15) for overlaying water, from 88.8 to 98.3% for pore water and from 71.2 to 105.2% for sediment for both active ingredients. The corresponding relative standard deviation (RSD) values were below 20%.

The specificity of the method was assured by MS/MS-detection and the absence of interfering peaks. The recovery and precision data show that the method meets the requirements of the guidance document SANTE/2020/12830, Rev.1; all criteria are fulfilled:

- control values do not exceed 30% of the method LOQ,
- mean recoveries at each level are in the range 70-120%,
- the RSD is < 20% per level.

Analysis results of Aclonifen in test samples (overlaying water)

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 days fresh		7 days spent	
1	0.000	2	nd	-	nd	-
2	3.293	2	2.679	81.4	2.262	68.7
3	10.56	2.667	10.48	99.3	7.989	75.7
4	33.78	8	29.03	86.0	26.18	77.5
5	107.9	13.33	123.7	114.6	80.64	74.7
6	345.8	40	303.4	87.7	290.1	83.9
7	1107	100	1273	115.1	952.4	86.1
			7 days fresh		14 days spent	
1	0.000	2	nd	-	nd	-
2	3.297	2	2.701	81.9	2.464	74.7
3	10.56	2.667	12.10	114.6	7.106	67.3
4	33.77	8	38.23	113.2	26.48	78.4
5	108.1	13.33	113.4	104.9	91.52	84.6
6	345.8	40	361.3	104.5	311.8	90.2
7	1107	100	1247	112.7	858.7	77.6

nd – Aclonifen was not detected; LOQ = 0.8052 µg/L; LOD = 0.2346 µg/L (regarding $DF_{analytical}$ = 1)

Analysis results of Flufenacet in test samples (overlaying water)

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 days fresh		7 days spent	
1	0.000	2	nd	-	< LOD	-
2	0.3728	2	0.3441	92.3	0.3316	88.9
3	1.195	2.667	1.173	98.2	1.064	89.1
4	3.824	8	3.775	98.7	3.353	87.7
5	12.22	13.33	13.10	107.2	11.62	95.1
6	39.15	40	40.76	104.1	37.84	96.6
7	125.3	100	131.5	105.0	121.6	97.0
			7 days fresh		14 days spent	
1	0.000	2	nd	-	< LOD	-
2	0.3732	2	0.3320	89.0	0.3427	91.8
3	1.195	2.667	1.187	99.4	1.105	92.5
4	3.823	8	3.673	96.1	3.617	94.6
5	12.24	13.33	12.30	100.4	11.81	96.5
6	39.15	40	39.82	101.7	37.17	94.9
7	125.3	100	127.1	101.5	119.3	95.3

nd – Flufenacet was not detected; LOQ = 0.09116 µg/L; LOD = 0.02635 µg/L (regarding $DF_{analytical}$ = 1)

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 48 AAL 0017 (21 35 CRA 0039) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 2.545 µg/L for Aclonifen and 0.2881 µg/L for Flufenacet. LOD was 0.7407 µg/L for Aclonifen and 0.08351 µg/L for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Effects of GLOB1310aH on *Desmodesmus subcapitatus* in an algal growth inhibition test. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Juckeland, D., 2021d. Report 21 48 AAL 0017 (21 35 CRA 0039). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania. All reported concentrations are pure active ingredients, i.e. they were corrected for purity.

An external matrix-matched calibration with the analytical reference items including 8 calibration levels was performed.

Calibration data Aclonifen
Błąd! Nie można odnaleźć źródła odwołania.

Calibration data Flufenacet

Błąd! Nie można odnaleźć źródła odwołania.*Method validation data*
Błąd! Nie można odnaleźć źródła odwołania.

Method validation results Aclonifen

Validation level	Nominal concentration [µg/L]	Analysed concentration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	nd nd	-	-	-
Low (LOQ)	2.545	2.699 2.504 2.506 2.415 2.489	2.523	99.1	4.2
High	72.72	81.82 76.26 70.11 74.52	74.84	102.9	6.1

		71.51			

nd – Aclonifen was not detected; LOQ = 2.545 µg/L; LOD = 0.7407 µg/L

Method validation results Flufenacet

Validation level	Nominal concentration [µg/L]	Analysed con-centration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	-0.006705 -0.006342	-	< LOD < LOD	-
Low (LOQ)	0.2881	0.2999 0.2901 0.2921 0.2934 0.2866	0.2924	101.5	1.7
High	8.232	8.555 8.744 8.479 8.749 8.645	8.635	104.9	1.4

nd – Flufenacet was not detected; LOQ = 0.2881 µg/L; LOD = 0.08351 µg/L

Concentrations of the target analytes in the blanks were < of its respective method LOD (i.e. 0.7407 µg/L for Aclonifen and 0.08351 µg/L Flufenacet).

The mean recovery values ranged from 99.1 to 104.9%. The corresponding relative standard deviation (RSD) values were below 20%.

The specificity of the method was assured by MS/MS-detection and the absence of interfering peaks. The recovery and precision data show that the method meets the requirements of the guidance document SANTE/2020/12830, Rev.1; all criteria are fulfilled:

- control values do not exceed 30% of the method LOQ,
- mean recoveries at each level are in the range 70-120%,
- the RSD is < 20% per level

Analysis results of Aclonifen in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		72 h spent	
1	0.000	2	< LOD	-	nd	-
2[1]	10.54	2	6.809	64.6	5.927	56.2
3[1]	18.98	3.571	13.04	68.7	14.10	74.3
4[1]	34.16	4	26.23	76.8	27.21	79.7
5	61.49	7.143	50.41	82.0	52.14	84.8
6	110.7	10	89.03	80.5	105.0	94.9

nd – Aclonifen was not detected; LOQ = 2.545 µg/L; LOD = 0.7407 µg/L

Analysis results of Flufenacet in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		72 h spent	
1	0.000	2	< LOD	-	< LOD	-

[1] Results are mean of first sample and retain sample

2[2]	1.194	2	1.132	94.8	0.9160	76.7
3[2]	2.148	3.571	2.137	99.5	1.981	92.2
4[2]	3.867	4	3.865	99.9	3.720	96.2
5	6.961	7.143	7.147	102.7	7.094	101.9
6	12.53	10	13.35	106.6	13.28	106.0

LOQ = 0.2881 µg/L; LOD = 0.08351 µg/L

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 48 AAL 0001 (21 35 CRA 0002) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 1.078 µg/L for Aclonifen and 0.1220 µg/L for Flufenacet. LOD was 0.3070 µg/L for Aclonifen and 0.03482 µg/L for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Effects of GLOB1310aH on *Pseudokirchneriella subcapitata* in an algal growth inhibition test. Verification of the concentration of Aclonifen and Flufenacet in the test solutions. Juckeland, D., 2021e. Report 21 48 AAL 0001 (21 35 CRA 0002). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania. All reported concentrations are pure active ingredients, i.e. they were corrected for purity.

An external matrix-matched calibration with the analytical reference items including 8 calibration levels was performed.

Calibration data Aclonifen
Błąd! Nie można odnaleźć źródła odwołania.

Calibration data Flufenacet

Błąd! Nie można odnaleźć źródła odwołania.*Method validation data*
Błąd! Nie można odnaleźć źródła odwołania.

Method validation results Aclonifen

Validation level	Nominal concentration [µg/L]	Analysed concentration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	nd nd	-	-	-

[2] Results are the mean of first sample and retain

Low (LOQ)	1.078	1.142 1.072 1.065 1.158 1.098	1.107	102.7	3.7
High	67.37	73.42 76.42 77.44 73.62 nd*	75.23	111.7	2.7

nd – Aclonifen was not detected; LOQ = 1.078 µg/L; LOD = 0.3070 µg/L; *Grubbs test showed it was an outlier, following calculation was without the outlier

Method validation results Flufenacet

Validation level	Nominal concentration [µg/L]	Analysed con-centration [µg/L]	Mean analysed concentration [µg/L]	Mean recovery [% of nominal concentration]	RSD [%]
Blank	0.000	-0.0100 -0.0114	-	< LOD < LOD	-
Low (LOQ)	0.1220	0.1337 0.1263 0.1211 0.1237 0.1192	0.1248	102.3	4.5
High	7.626	8.479 8.555 8.422 8.478 nd*	8.483	111.2	0.6

nd – Flufenacet was not detected; LOQ = 0.1220µg/L; LOD = 0.03482 µg/L; *Grubbs test showed it was an outlier, following calculation was without the outlier

– **Błąd! Nie można odnaleźć źródła odwołania.**

Błąd! Nie można odnaleźć źródła odwołania.

Analysis results of Aclonifen in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		72 h spent	
1	0.000	2	nd	-	nd	-
2	4.347	2	3.862	88.8	2.726	62.7
3	9.560	2	7.659	80.1	7.537	78.8
4	21.03	4	20.44	97.2	17.55	83.4
5	46.27	4	48.35	104.5	37.16	80.3
6	101.8	8	106.6	104.7	82.01	80.6

nd – Aclonifen was not detected; LOQ = 1.078 µg/L; LOD = 0.3070 µg/L

Analysis results of Flufenacet in test samples

Treatment	Nominal concentration [µg/L]	Dilution factor	Analysed concentration [µg/L]	Recovery [% of nominal]	Analysed concentration [µg/L]	Recovery [% of nominal]
			0 h fresh		72 h spent	
1	0.000	2	< LOD	-	< LOD	-
2	0.4921	2	0.4760	96.7	0.4309	87.6
3	1.082	2	1.077	99.5	1.008	93.2
4	2.381	4	2.367	99.4	2.533	106.4
5	5.238	4	5.198	99.2	5.388	102.9
6	11.53	8	11.50	99.8	11.83	102.7

LOQ = 0.1220µg/L; LOD = 0.03482 µg/L

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 48 BBA 0001 (21 35 CRA 006) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. Validation summary – contact toxicity test: LOQ was 47367 mg/L for Aclonifen and 5362 mg/L for Flufenacet. Validation results – oral toxicity test: LOQ was 1341 mg/kg for Aclonifen and 152 mg/kg for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Acute toxicity of GLOB1310aH to the bumblebee *Bombus terrestris* L. under laboratory conditions Determination of the concentration of aclonifen and flufenacet in feeding solutions and test solutions by LC-MS/MS., Amsel, K., 2021. Report 21 48 BBA 0001 (21 35 CRA 0006). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania.

Validation summary

Validation results – contact toxicity test

Sample description	*Number of replicates*	*Nominal conc. of Aclonifen [mg/L]*	*Mean analysed conc. of Aclonifen [mg/L]*	*Mean recovery [% of nominal]*	*RSD [%]*
21CRB0006-Val-high-con	5	118417	114106	96.4	2.23
21CRB0006-Val-low-con	5	47367	46333	97.8	2.27

21CRB0006 Val-blank-con	2	0.000	< 30% LOQ	-	-

LOQ = 47367 mg/L of Aclonifen

Sample description	*Number of replicates*	*Nominal conc. of Flufenacet [mg/L]*	*Mean analysed conc. of Flufenacet [mg/L]*	*Mean recovery [% of nominal]*	*RSD [%]*
21CRB0006-Val-high-con	5	13405	14023	105	1.87
21CRB0006-Val-low-con	5	5362	5391	101	0.726
21CRB0006 Val-blank-con	2	0.000	< 30% LOQ	-	-

LOQ = 5362 mg/L of Flufenacet

Validation results – oral toxicity test

Sample description	*Number of replicates*	*Nominal conc. of Aclonifen [mg/kg]*	*Mean analysed conc. of Aclonifen [mg/kg]*	*Mean recovery [% of nominal]*	*RSD [%]*
21CRB0006-Val-high-oral	5	11564	10418	90.1	2.89
21CRB0006-Val-low-oral	5	1341	1161	86.6	1.03
21CRB0006 Val-blank-oral	2	0.000	< 30% LOQ	-	-

LOQ = 1341 mg/kg of Aclonifen

Sample description	*Number of replicates*	*Nominal conc. of Flufenacet [mg/kg]*	*Mean analysed conc. of Flufenacet [mg/kg]*	*Mean recovery [% of nominal]*	*RSD [%]*
21CRB0006-Val-high-oral	5	1309	1108	85	4.23
21CRB0006-Val-low-oral	5	152	127	83	0.929
21CRB0006 Val-blank-oral	2	0.000	< 30% LOQ	-	-

LOQ = 152 mg/kg of Flufenacet

Comments of zRMS:	The analytical phase of the study 21 48 BBA 0001 (21 35 CRA 006) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. Validation summary – contact toxicity test: LOQ was 47367 mg/L for Aclonifen and 5362 mg/L for Flufenacet. Validation results – oral toxicity test: LOQ was 1341 mg/kg for Aclonifen and 152 mg/kg for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference: KCP 5.2

Report: Acute toxicity of GLOB1310aH to the bumblebee *Bombus terrestris* L. under laboratory conditions
Determination of the concentration of aclonifen and flufenacet in feeding solutions and test solutions by LC-MS/MS., Amsel, K., 2021. Report 21 48 BBA 0001 (21 35 CRA 0006). Biochem Agrar GmbH, Germany (filed in section B9)

Guideline(s): Yes, SANTE/2020/12830, Rev.1

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.

The recovery and precision data show that the influences of sample matrix were within the limits of the guidance document SANTE/2020/12830, Rev.1; all criteria were fulfilled:

- blank values did not exceed 30% of the lowest validated concentration,
- mean recoveries for each level were in the range 70-120%,
- the RSD was < 20% per level,
- linearity was given with correlation coefficients > 0.99.

Limit of Quantification

Błąd! Nie można odnaleźć źródła odwołania.

Analysis results

Measurement results of Aclonifen – contact toxicity test

Sample description	*Nominal* conc. of Aclonifen [mg/L]*	*Nominal* conc. of Aclonifen regarding $DF_{overall}$ [µg/L]*	*$DF_{overall}$*	*Measured conc. of Aclonifen [µg/L]*	*Analysed conc. of Aclonifen [mg/L]*	*Recovery [% of nominal]*
21BBA0001-BC-con	0.000	0.00	800000	0.000	<30%LOQ	-
21BBA0001-AT-con	90617	113	800000	103	85132	93.9

LOQ =47367 mg/L of Aclonifen (corresponding to 59.2 µg/L regarding $DF_{overall}$ of lowest validation level)
*Nominal conc. is based on analysed content of a.i. according to certificate of analysis.

The recovery of Aclonifen was 93.9% for the test solution. In the control sample, the concentration of the active ingredient was below 30% of LOQ.

Measurement results of Flufenacet – contact toxicity test

Sample description	*Nominal* conc. of Flufenacet [mg/L]*	*Nominal* conc. of Flufenacet regarding $DF_{overall}$ [µg/L]*	*$DF_{overall}$*	*Measured conc. of Flufenacet [µg/L]*	*Analysed conc. of Flufenacet [mg/kg]*	*Recovery [% of nominal]*
21BBA0001-BC-con	0.000	0.00	800000	0.000	<30%LOQ	-
21BBA0001-AT-con	10258	12.8	800000	12.3	10579	103

LOQ =5362 mg/L of Flufenacet (corresponding to 6.70 µg/L regarding $DF_{overall}$ of lowest validation level)
*Nominal conc. is based on analysed content of a.i. according to certificate of analysis.

The recovery of Flufenacet was 103% for the test solution. In the control sample, the concentration of the active ingredient was below 30% of LOQ.

Measurement results of Aclonifen – oral toxicity test

Sample description	*Nominal* conc. of Aclonifen [mg/kg]*	*Nominal* conc. of Aclonifen regarding $DF_{overall}$ [µg/L]*	*$DF_{overall}$*	*Measured conc. of Aclonifen [µg/L]*	*Analysed conc. of Aclonifen [mg/kg]*	*Recovery [% of nominal]*
21BBA0001-AC-oral	0.000	0.000	50	0.000	<30%LOQ	-
21BBA0001-AT-oral	8680	130	66667	132	8804	101
21BBA0001-BT-oral	6423	128	50000	121	6055	94.3
21BBA0001-CT-oral	4753	143	33333	133	4439	93.4
21BBA0001-DT-oral	3517	141	25000	136	3351	95.3
21BBA0001-ET-oral	2603	156	16667	151	2479	95.2

LOQ =1341 mg/kg of Aclonifen (corresponding to 80.5 µg/L regarding $DF_{overall}$ of lowest validation level)
*Nominal conc. is based on analysed content of a.i. according to certificate of analysis.

The recoveries of Aclonifen were between 93.4% and 101% for the feeding solutions. In the control sample, the concentration of the active ingredient was below 30% of LOQ.

Measurement results of Flufenacet – oral toxicity test

Sample description	*Nominal* conc. of Flufenacet [mg/kg]*	*Nominal* conc. of Flufenacet regarding $DF_{overall}$ [µg/L]*	*$DF_{overall}$*	*Measured conc. of Flufenacet [µg/L]*	*Analysed conc. of Flufenacet [mg/kg]*	*Recovery [% of nominal]*
21BBA0001-AC-oral	0.000	0.000	50	0.000	<30%LOQ	-
21BBA0001-AT-oral	983	14.7	66667	14.3	988	101
21BBA0001-BT-oral	727	14.5	50000	13.4	698	96.0
21BBA0001-CT-oral	538	16.1	33333	15.1	521	96.9
21BBA0001-DT-oral	398	15.9	25000	14.8	386	96.8
21BBA0001-ET-oral	295	17.7	16667	16.7	290	98.5

LOQ =152 mg/kg of Flufenacet (corresponding to 9.11 µg/L regarding $DF_{overall}$ of lowest validation level)
*Nominal conc. is based on analysed content of a.i. according to certificate of analysis.

The recoveries of Flufenacet were between 96.0% and 101 % for the feeding solutions. In the control sample, the concentration of the active ingredient was below 30% of LOQ.

Comments of zRMS:	The analytical phase of the study 21 48 BAC 0004 (21 35 CRB 0004) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 13.4 mg/kg for Aclonifen and 1.52 mg/kg for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference: KCP 5.2

Report: Chronic toxicity of GLOB1310aH to the honey bee *Apis mellifera* L. under laboratory conditions. Determination of the concentrations of aclonifen and flufenacet in feeding solutions by LC-MS/MS., Dreßler, K., 2021. Report 21 48 BAC 0004 (21 35 CRB 0004). Biochem Agrar GmbH, Germany

(filed in section B9)

Guideline(s): Yes, SANTE/2020/12830, Rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania.*Summary of method validation results:*

Mass transition	*Sample description*	*Number of replicates*	*Nominal conc. of aclonifen [mg/kg]*	*Mean analysed conc. of aclonifen [mg/kg]*	*Mean recovery [% of nominal]*	*RSD [%]*
	21CRB0004-Val-high	5	2980	2986	103	1.41
m/z 265 -> 248	21CRB0004-Val-low	5	13.4	12.8	95.2	1.69
	21CRB0004-Val-blank	2	0.000	< 30% of LOQ	-	-

LOQ = 13.4 mg/kg of aclonifen

Mass transition	*Sample description*	*Number of replicates*	*Nominal conc. of flufenacet [mg/kg]*	*Mean analysed conc. of flufenacet [mg/kg]*	*Mean recovery [% of nominal]*	*RSD [%]*
	21CRB0004-Val-high	5	337	349	104	1.73
m/z 364 -> 152	21CRB0004-Val-low	5	1.52	1.52	100	2.37
	21CRB0004-Val-blank	2	0.000	< 30% of LOQ	-	-

LOQ = 1.52 mg/kg of flufenacet

Błąd! Nie można odnaleźć źródła odwołania.Błąd! Nie można odnaleźć źródła odwołania.

The high validation levels were 2980 mg/kg of aclonifen and 337 mg/kg of flufenacet, corresponding to 179 µg/L aclonifen and 20.2 µg/L flufenacet regarding the applied dilution factor for the validation high samples.

Analysis results

Analysis results for aclonifen of samples from the biological phase of the study

Sample identification	*Nominal conc. of aclonifen [mg/kg]*	*Analysed conc. of aclonifen [mg/kg]*	*Recovery [% of nominal]*
21BAC0004-D0-BC-A2	0.000	< 30% LOQ	-
21BAC0004-D1-BC-A2		< 30% LOQ	-
21BAC0004-D2-BC-A2		< 30% LOQ	-
21BAC0004-D3-BC-A2		< 30% LOQ	-
21BAC0004-D4-BC-A2		< 30% LOQ	-
21BAC0004-D5-BC-A2		< 30% LOQ	-
21BAC0004-D6-BC-A2		< 30% LOQ	-
21BAC0004-D7-BC-A2		< 30% LOQ	-
21BAC0004-D8-BC-A2		< 30% LOQ	-
21BAC0004-D9-BC-A2		< 30% LOQ	-
21BAC0004-D0-GT-A2	26.2	26.1	99.5
21BAC0004-D1-GT-A2		26.4	101
21BAC0004-D2-GT-A2		24.1	92.0
21BAC0004-D3-GT-A2		26.1	99.5

21BAC0004-D4-GT-A2	2254	23.5	89.4
21BAC0004-D5-GT-A2		25.1	95.7
21BAC0004-D6-GT-A2		25.1	95.6
21BAC0004-D7-GT-A2		26.4	101
21BAC0004-D8-GT-A2		23.5	89.7
21BAC0004-D9-GT-A2		25.4	96.8
21BAC0004-D0-AT-A2		2244	99.6
21BAC0004-D1-AT-A2		2260	100
21BAC0004-D2-AT-A2		2301	102
21BAC0004-D3-AT-A2		2266	101
21BAC0004-D4-AT-A2		2311	103
21BAC0004-D5-AT-A2		2174	96.4
21BAC0004-D6-AT-A2		2202	97.7
21BAC0004-D7-AT-A2		2184	96.9
21BAC0004-D8-AT-A2		2231	99.0
21BAC0004-D9-AT-A2		225	100

LOQ = 13.4 mg/kg of aclonifen (corresponding to 67.1 µg/L regarding DF of lowest validation level)

Analysis results for flufenacet of samples from the biological phase of the study

Sample identification	*Nominal conc. of flufenacet [mg/kg]*	*Analysed conc. of flufenacet [mg/kg]*	*Recovery [% of nominal]*
21BAC0004-D0-BC-A2	0.000	< 30% LOQ	-
21BAC0004-D1-BC-A2		< 30% LOQ	-
21BAC0004-D2-BC-A2		< 30% LOQ	-
21BAC0004-D3-BC-A2		< 30% LOQ	-
21BAC0004-D4-BC-A2		< 30% LOQ	-
21BAC0004-D5-BC-A2		< 30% LOQ	-
21BAC0004-D6-BC-A2		< 30% LOQ	-
21BAC0004-D7-BC-A2		< 30% LOQ	-
21BAC0004-D8-BC-A2		< 30% LOQ	-
21BAC0004-D9-BC-A2		< 30% LOQ	-
21BAC0004-D0-GT-A2	2.97	2.99	101
21BAC0004-D1-GT-A2		3.16	106
21BAC0004-D2-GT-A2		2.87	96.7
21BAC0004-D3-GT-A2		3.03	102
21BAC0004-D4-GT-A2		2.70	90.8
21BAC0004-D5-GT-A2		2.93	98.5
21BAC0004-D6-GT-A2		2.96	99.7
21BAC0004-D7-GT-A2		3.04	102
21BAC0004-D8-GT-A2		2.87	96.6
21BAC0004-D9-GT-A2		3.00	101
21BAC0004-D0-AT-A2	255	259	101
21BAC0004-D1-AT-A2		264	104
21BAC0004-D2-AT-A2		264	103
21BAC0004-D3-AT-A2		260	102
21BAC0004-D4-AT-A2		270	106
21BAC0004-D5-AT-A2		252	98.6
21BAC0004-D6-AT-A2		257	101
21BAC0004-D7-AT-A2		250	98.0
21BAC0004-D8-AT-A2		261	102
21BAC0004-D9-AT-A2		263	103

LOQ = 1.52 mg/kg of flufenacet (corresponding to 7.59 µg/L regarding DF of lowest validation level)

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 48 BLC 0003 (21 35 CRB 0005) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 5.31 mg/kg for Aclonifen and 0.601 mg/kg for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	GLOB1310aH-Repeated exposure to the honey bee (*Apis mellifera*) L. larvae under laboratory conditions. Determination of the concentrations of aclonifen and flufenacet in final diets by LC-MS/MS., Hänsel, M., 2021. Report 21 48 BLC 0003 (21 35 CRB 0005). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania.*Summary of method validation results:*

Mass transition	*Sample description*	*Number of replicates*	*Nominal conc. of aclonifen [mg/kg]*	*Mean analysed conc. of aclonifen [mg/kg]*	*Mean recovery [% of nominal]*	*RSD [%]*
	21CRB0005-Val-high	5	221	202	91.4	1.28
m/z 265 -> 248	21CRB0005-Val-low	5	5.31	4.44	83.6	3.64
	21CRB0005-Val-blank	2	0.000	< 30% of LOQ	-	-

LOQ = 5.31 mg/kg of aclonifen

Mass transition	*Sample description*	*Number of replicates*	*Nominal conc. of flufenacet [mg/kg]*	*Mean analysed conc. of flufenacet [mg/kg]*	*Mean recovery [% of nominal]*	*RSD [%]*
	21CRB0005-Val-high	5	25.04	23.4	93.5	2.65
m/z 364 -> 152	21CRB0005-Val-low	5	0.601	0.528	87.9	2.03
	21CRB0005-Val-blank	2	0.000	< 30% of LOQ	-	-

LOQ = 0.601 mg/kg of flufenacet

Błąd! Nie można odnaleźć źródła odwołania.Błąd! Nie można odnaleźć źródła odwołania.

The high validation levels were 221 mg/kg of aclonifen and 25.04 mg/kg of flufenacet, corresponding to 133 µg/L aclonifen and 15.0 µg/L flufenacet regarding the applied dilution factor for the validation high samples.

Analysis results

Analysis results for aclonifen of samples from the biological phase of the study

Sample identification	*Nominal conc. of aclonifen [mg/kg]*	*Analysed conc. of aclonifen [mg/kg]*	*Recovery [% of nominal]*
21BLC0003-D3-AC-A	0.000	< 30% LOQ	-
21BLC0003-D4-AC-A		< 30% LOQ	-
21BLC0003-D5-AC-A		< 30% LOQ	-
21BLC0003-D6-AC-A		< 30% LOQ	-
21BLC0003-D3-ET-A	10.3	6.60	63.8
21BLC0003-D4-ET-A		9.23	89.2
21BLC0003-D5-ET-A		8.10	78.3
21BLC0003-D6-ET-A		9.22	89.1
21BLC0003-D3-DT-A	20.7	13.3	64.2
21BLC0003-D4-DT-A		18.2	87.9
21BLC0003-D5-DT-A		16.2	78.3
21BLC0003-D6-DT-A		14.8	71.7
21BLC0003-D3-CT-A	41.4	34.4	83.2
21BLC0003-D4-CT-A		36.0	86.9
21BLC0003-D5-CT-A		31.8	76.8
21BLC0003-D6-CT-A		33.9	81.9
21BLC0003-D3-BT-A	82.8	74.3	89.8
21BLC0003-D4-BT-A		70.1	84.7
21BLC0003-D5-BT-A		67.3	81.3
21BLC0003-D6-BT-A		68.4	82.7
21BLC0003-D3-AT-A	166	139	83.8
21BLC0003-D4-AT-A		141	85.5
21BLC0003-D5-AT-A		124	75.2
21BLC0003-D6-AT-A		127	77.0

LOQ = 5.31 mg/kg of aclonifen (corresponding to 63.7 µg/L regarding DF of lowest validation level)

Analysis results for flufenacet of samples from the biological phase of the study

Sample identification	*Nominal conc. of flufenacet [mg/kg]*	*Analysed conc. of flufenacet [mg/kg]*	*Recovery [% of nominal]*
21BLC0003-D3-AC-A	0.000	< 30% LOQ	-
21BLC0003-D4-AC-A		< 30% LOQ	-
21BLC0003-D5-AC-A		< 30% LOQ	-
21BLC0003-D6-AC-A		< 30% LOQ	-
21BLC0003-D3-ET-A	1.17	1.30	111
21BLC0003-D4-ET-A		1.19	102
21BLC0003-D5-ET-A		1.06	90.8
21BLC0003-D6-ET-A		1.13	96.3
21BLC0003-D3-DT-A	2.34	2.57	110
21BLC0003-D4-DT-A		2.42	104
21BLC0003-D5-DT-A		2.24	95.5
21BLC0003-D6-DT-A		2.06	87.9
21BLC0003-D3-CT-A	4.68	4.82	103
21BLC0003-D4-CT-A		4.41	94.2
21BLC0003-D5-CT-A		4.12	88.0

21BLC0003-D6-CT-A		4.25	90.8
21BLC0003-D3-BT-A	9.37	9.73	104
21BLC0003-D4-BT-A		9.31	99.4
21BLC0003-D5-BT-A		8.64	92.3
21BLC0003-D6-BT-A		8.09	86.3
21BLC0003-D3-AT-A	18.7	17.6	93.8
21BLC0003-D4-AT-A		17.5	93.5
21BLC0003-D5-AT-A		16.7	88.9
21BLC0003-D6-AT-A		15.3	81.7

LOQ = 0.601 mg/kg of flufenacet (corresponding to 7.21 µg/L regarding DF of lowest validation level)

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 46 PSE 0001 (21 35 CRP 0002) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 7.100 mg/L for Aclonifen and 0.8038 mg/L for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Effects of GLOB1310aH on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions Verification of the concentration of Aclonifen and Flufenacet in the spray solution specimens., Friedeman, M., 2021. Report 21 46 PSE 0001 (21 35 CRP 0002). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania.

An external matrix-matched calibration with the analytical reference items including 5 calibration levels was performed.

*Calibration data for **Błąd! Nie można odnaleźć źródła odwołania.***

Błąd! Nie można odnaleźć źródła odwołania.

*Calibration data for **Błąd! Nie można odnaleźć źródła odwołania.***

Błąd! Nie można odnaleźć źródła odwołania.
Method validation data

Błąd! Nie można odnaleźć źródła odwołania.

*Method verification for **Błąd! Nie można odnaleźć źródła odwołania.*****Błąd! Nie można odnaleźć źródła odwołania.**

*Method verification for **Błąd! Nie można odnaleźć źródła odwołania.*****Błąd! Nie można odnaleźć źródła odwołania.**

The recovery values were in the range of 70 %–110 % with relative standard deviations (RSD %) ≤10 %, which demonstrates acceptable accuracy and precision of the method.
Błąd! Nie można odnaleźć źródła odwołania.

Analytical results

*Summary of **Błąd! Nie można odnaleźć źródła odwołania.** in the spray solution specimen*

Błąd! Nie można odnaleźć źródła odwołania.

Błąd! Nie można odnaleźć źródła odwołania.

*Summary of **Błąd! Nie można odnaleźć źródła odwołania.** in the spray solution specimen*

Błąd! Nie można odnaleźć źródła odwołania.

Błąd! Nie można odnaleźć źródła odwołania.

Conclusion

Błąd! Nie można odnaleźć źródła odwołania.

Comments of zRMS:	The analytical phase of the study 21 46 PVV 0001 (21 35 CRP 0001) is acceptable and suitable for the determination of the active ingredients Aclonifen and Flufenacet in the test solutions. LOQ was 7.100 mg/L for Aclonifen and 0.8038 mg/L for Flufenacet. The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830, Rev.1.

Reference:	KCP 5.2
Report	Effects of GLOB1310aH on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions Verification of the concentration of Aclonifen and Flufenacet in the spray solution specimens., Friedeman, M., 2021. Report 21 46 PVV 0001 (21 35 CRP 0001). Biochem Agrar GmbH, Germany (filed in section B9)
Guideline(s):	Yes, SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

Błąd! Nie można odnaleźć źródła odwołania.

An external matrix-matched calibration with the analytical reference items including 5 calibration levels was performed.

*Calibration data for **Błąd! Nie można odnaleźć źródła odwołania.***

Błąd! Nie można odnaleźć źródła odwołania.

*Calibration data for **Błąd! Nie można odnaleźć źródła odwołania.***

Błąd! Nie można odnaleźć źródła odwołania.
Method validation data

Błąd! Nie można odnaleźć źródła odwołania.

*Method validation for **Błąd! Nie można odnaleźć źródła odwołania.*****Błąd! Nie można odnaleźć źródła odwołania.**

*Method validation for **Błąd! Nie można odnaleźć źródła odwołania.*** **Błąd! Nie można odnaleźć źródła odwołania.**

The recovery values were in the range of 70 %–110 % with relative standard deviations (RSD %) ≤10 %, which demonstrates acceptable accuracy and precision of the method.
Błąd! Nie można odnaleźć źródła odwołania.

Analytical results

*Summary of **Błąd! Nie można odnaleźć źródła odwołania.** in the spray solution specimen*

Błąd! Nie można odnaleźć źródła odwołania.

Błąd! Nie można odnaleźć źródła odwołania.

*Summary of **Błąd! Nie można odnaleźć źródła odwołania.** in the spray solution specimen*

Błąd! Nie można odnaleźć źródła odwołania.

Błąd! Nie można odnaleźć źródła odwołania.

Conclusion

Błąd! Nie można odnaleźć źródła odwołania.

A 2.2 Analytical methods for flufenacet

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.2.2.1.1 Analytical method 1

No new or additional studies have been submitted

A 2.2.2.1.1.1 Independent laboratory validation

No new or additional studies have been submitted

A 2.2.2.1.1.2 Confirmatory method

No confirmatory method is required

A 2.2.2.1.1.3 Extraction efficiency

No new or additional studies have been submitted

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.2.2.4.1 Analytical method 1

A 2.2.2.4.1.1 Method validation

Comments of zRMS:	Validation of an Analytical Method for the Determination of Flufenacet and its metabolites (M1, M2, M5, M7, M9, trifluoroacetic acid and trifluoroethanesulfonic acid) in surface and drinking water. Merdian, H., 2017. Study S15-04126. The methods were found to be valid according to the guidance document SANCO/825/00 rev 8.1, for the determination of all analytes in water (surface and drinking) with the tested LOQ of 0.10 µg/L for flufenacet, M1, M2, M5, M7, M9 and trifluoroethanesulfonic acid, and the tested LOQ of 3.1 µg/L for trifluoroacetic acid in surface water and 0.65 µg/L for trifluoroacetic acid in drinking water. This submitted study has been validated in a proper manner.

Reference:	KCA 5.2
Report	Validation of Analytical Method for the Determination of flufenacet and its metabolites (M1, M2, M5, M7, M9, trifluoroacetic acid and trifluoroethanesulfonic acid) in surface and drinking water. Merdian, H., 2017. Study S15-04126. Eurofins Agroscience Services Chem, UK
Guideline(s):	Yes, SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and Methods

The objective of the study was to validate analytical methods as described in this study for the determination of flufenacet and its metabolites (M1, M2, M5, M7, M9, trifluoroacetic acid (TFA) and trifluoroethanesulfonic acid (TFESA)) in two different waters (surface and drinking) in accordance to the guidance document SANCO/825/00, rev. 8.1, with an intended limit of quantification of 0.10 µg/L for flufenacet, M1, M2, M5, M7, M9 and trifluoroethanesulfonic acid, and 3.1 µg/L (surface water) and 0.65 µg/L (drinking water) for trifluoroacetic acid (TFA). All analytical methods for all analytes have been validated in two different waters (surface and drinking).

Quantification of all analytes was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated per matrix/analyte in order to demonstrate that the methods achieved a high level of selectivity

-Method summary:

Extraction: An aliquot of each sample was taken and then filtered using a syringe filter
Detection: Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
LOQ: 0.10 µg/L for each analyte

-Mobile phases:

Phase A: 0.1% formic acid in water
Phase B: 0.1% formic acid in methanol
Water/methanol/formic acid in water (50/50/0/1, v/v)

-Chromatographic conditions

HPLC system: Agilet 1200 Series or Shimadzu Nexera X2 LC-30AD HPLC and SIL-30ACMP auto-sampler
Column: Phenomenex Kinetex PFP, 100 mm length, 3 mm diameter, 2.6 µm particle size
Column temperature: 50°C
Injection volume: 20µL or 50µL
Mobile phases: Eluent A: water containing 0/1% formic acid
Eluent B: methanol containing 0.1% formic acid
Gradient:

Time (min)	%Eluent A	%Eluent B	Flow (µL/min)
0.00	80	20	600
3.5	10	90	600
8.5	10	90	600
8.6	80	20	600
12	80	20	600

Retention time: Flufenacet approx. 5.5 min
M1 approx. 4.0 min
M2 approx. 3.4 min
M5 approx. 4.9 min
M7 approx. 4.0 min
M9 approx. 4.2 min

-Mass spectrometric conditions

MS system: AB Sciex 5500 QTrap LC/MS/MS System
Ionisation type: Turbo Ion Spray
Polarity: Positive Ion Mode (Flufenacet, M1, M5, M7)
Negative Ion Mode (M2 and M9)
Scan type: Multiple Reaction Monitoring
Capillary voltage: 5500 V (Flufenacet, M1, M5, M7)
-4500 (M2, M9)
Ionspray turbo heater: 500°C
Curtain gas: 35 Gas Flow: 35
Collision gas: 10 Gas Flow: 55

Analyte monitored	Ion mass transition monitored *(m/z)*	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
Flufenacet	364.0 → 194.2 #	71	10	15	12	50
	364.0 → 152.0	71	10	29	10	50
M1	226.1→138.1 #	51	10	21	10	150
	226.1→ 109.9	51	10	37	10	150
M2	274.1→ 120.9 #	-90	-10	-28	-9	50
	274.1→ 79.9	-90	-10	-54	-7	50
M5	242.1→ 154.1 #	16	10	19	14	50
	242.1→ 112.0	16	10	27	10	50
M7	274.1→ 232.1 #	51	10	17	16	50
	274.1→ 112.0	51	10	31	8	50
M9	168.8→ 112.9 #	-45	-10	-22	-9	50
	168.8→ 109.0	-45	-10	-24	-7	50

proposed (and/or used) for quantification but both of the mass transitions listed can be used for quantification

Results

Selectivity
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of water (surface and drinking) for flufenacet and its metabolites (M1, M2, M5, M7, M9 and trifluoroethanesulfonic acid), so that a high level of selectivity was demonstrated.

Matrix effects
Matrix suppression or enhancement was < 20 % for both matrices for flufenacet, M1, M2, M5, M7 and M9 (quantification ion only) and thus deemed to be insignificant. However, matrix suppression or enhancement was ≥ 20 % for M9 in drinking water (confirmation ion only) and matrix-matched standards were used for all analytes, except for M5 where solvent standards were used, for quantification to compensate for any matrix effects. Since no matrix effect was detected for M5 for both transitions solvent standards were used for evaluation of results this does not have any impact on the validation results and is fully acceptable. Matrix suppression was > 20 % for TFESA and TFA for both matrices and matrix-matched standards were used for quantification to compensate for any matrix effects

Linearity
The linearity of the detector response was demonstrated by single determination of either matrix-matched or solvent calibration standards at a minimum of five (5) concentration levels ranging from 0.03 ng/mL to 5.0 ng/mL for both matrices (flufenacet and its metabolites M1, M2, M5, M7 and M9), 1.5 ng/mL to 25 ng/mL for surface water or 60 ng/mL for drinking water (TFESA) and 1.95 ng/mL to 100 ng/mL (TFA) in surface and drinking water. These ranges correspond to a fortification level of 0.03 µg/L to 5.0 µg/L (flufenacet and its metabolites M1, M2, M5, M7 and M9), 0.03 µg/L to 0.50 µg/L (for surface water) or 1.2 µg/L (for drinking water) (TFESA) and 0.195 µg/L to 10 µg/L (TFA). This covers the range from no more than 30 % of the limit of quantification (LOQ) and at least + 20 % of the expected highest analyte concentration in the final sample extract.
The calibration curves obtained for both mass transitions and for all analytes/matrices had correlation coefficients (R) ≥ 0.995
Accuracy and precision
Accuracy was determined by fortification of control samples of drinking and surface water, with known amounts of the test items at the LOQ and at ten times the LOQ, and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined as the relative standard deviation of the recoveries at each fortification level. Flufenacet, M1, M2, M5, M7 and M9 were fortified jointly and quantified separately, while trifluoroethanesulfonic acid (TFESA) and trifluoroacetic acid (TFA) were fortified and quantified separately.
All mean recovery values at the fortification levels of 0.10 µg/L and 1.0 µg/L for flufenacet, M1, M2, M5,

M7, M9 and TFESA, and 0.65 µg/L, 6.5 µg/L (drinking water), 3.1 µg/L and 31 µg/L (surface water) for TFA, for each mass transition comply with the standard acceptance criteria.

LOQ and LOD
The LOQ is the lowest validated fortification level for each analyte and was successfully established at 0.10 µg/L for flufenacet, M1, M2, M5, M7, M9 and trifluoroethanesulfonic acid in water (surface and drinking) for each mass transition, with the LOD set at 0.03 µg/L for all matrices, which is 30 % of the LOQ.

Stability of Stock Solutions
Flufenacet and its metabolites M1, M2, M5, M7 and M9 were found to be stable for at least 218 days when prepared in methanol and stored refrigerated (1 °C to 10 °C) in the dark. Trifluoroethanesulfonic acid and trifluoroacetic acid were found to be stable for at least 200 days when prepared in methanol/water (50/50, v/v) containing 0.1 % formic acid and stored refrigerated (1 °C to 10 °C) in the dark.

Stability of fortification solutions
Flufenacet and its metabolites M1, M2, M5, M7 and M9 were found to be stable for at least 45 days when prepared in water containing 0.1% formic acid and stored refrigerated (1 °C to 10 °C) in the dark. Trifluoroethanesulfonic acid and trifluoroacetic acid were found to be stable for at least 200 days when prepared in methanol/water (50/50, v/v) containing 0.1 % formic acid and stored refrigerated (1 °C to 10 °C) in the dark.

Extract stability
Flufenacet and its metabolites (M1, M2, M5, M7, M9, trifluoroethanesulfonic acid and trifluoroacetic acid) were found to be stable in final extracts of both matrices for at least 8 days when stored refrigerated (1 °C to 10 °C) in the dark.

Conclusions

The methods were found to be valid according to the guidance document SANCO/825/00 rev 8.1, for the determination of all analytes in water (surface and drinking) with the tested LOQ of 0.10 µg/L for flufenacet, M1, M2, M5, M7, M9 and trifluoroethanesulfonic acid, and the tested LOQ of 3.1 µg/L for trifluoroacetic acid in surface water and 0.65 µg/L for trifluoroacetic acid in drinking water.

A 2.2.2.4.1.2 Independent laboratory validation

No new or additional studies have been submitted

A 2.2.2.4.1.3 Confirmatory method

No confirmatory method is required

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP

5.2)

Comments of zRMS:	Validation of an Analytical Method for the Determination of Flufenacet and its metabolite M9 in Plasma; Asekunowo, J. 2019; Report P 4840 G is acceptable and suitable for the determination of the active ingredients Flufenacet and its metabolite M9 in plasma. The method validation of the analytical procedure was successfully completed and lead to recovery rates in the range from 70% to 110%. Residues of flufenacet in the blank control samples were ≤30 % of the LOQ. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1, 16/11/2010. This submitted study has been validated in a proper manner.

Reference:	KCP 4.2
Report	Development and Validation of an Analytical Method for the Determination of Flufenacet and its metabolite M9 in Plasma. Asekunowo, J. 2019. Report P 4840 G. EAG Laboratories, Germany
Guideline(s):	Yes, SANCO/3029/99 rev. 4 11/07/2000, SANCO/825/00 rev. 8.1, 16/11/2010
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Residues of flufenacet are extracted using methanol containing 1.5% formic acid. After addition of water and subsequent shaking, centrifugation and dilution, the final sample extract is analyzed for flufenacet by LC-MS/MS monitoring the following mass transitions:

Flufenacet	364 m/z -> 152 m/z (quantification)
	364 m/z -> 194 m/z (confirmation)

Results

The method validation of the analytical procedure was successfully completed and lead to recovery rates in the range from 70% to 110%. Residues of flufenacet in the blank control samples were ≤30 % of the LOQ.

The summary of the method validation are presented in the table below.

Matrix	Fortification (mg/L)	Flufenacet Metabolite M9			Flufenacet	
		Validation Results	169 m/z -> 113 m/z	169 m/z -> 109 m/z	364 m/z -> 152 m/z	364 m/z -> 194 m/z
Plasma	0.050 LOQ	Mean (n =5)	101%	102%	102%	104%
		RSD (n =5)	3%	4%	2%	1%
	0.50	Mean (n =5)	100%	100%	101%	105%
		RSD (n =5)	3%	4%	2%	3%
	Overall	Mean (n =10)	101%	101%	101%	104%
		RSD (n =10)	3%	4%	2%	2%
RSD: Relative Standard Deviation						

Conclusions
The method validation of an analytical method for the determination of flufenacet in plasma by LC-MS/MS was performed. It is concluded that the analytical method is suitable for the analysis of flufenacet in plasma. The method validation set conducted within this study was performed in compliance with the EC Guidance documents on residue analytical method SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1, 16/11/2010.

A 2.2.2.7 Other Studies/ Information

In several residue studies and ecotoxicological studies summarised in sections B7 and B9 of the dRR, analytical methods were used for the detection of the active substances Aclonifen and flufenacet in the different test mediums. The analytical part of these studies is summarised here.

Comments of zRMS:	The method Validation of Analytical Methods for the Determination of Flufenacet in Different Matrices of Plant Origin. Winter, O., Amann, S., 2021. Report S20-09167 (GLC-2019V) was successfully validated for the determination of flufenacet and its metabolites in wheat (grain and straw) with the tested LOQ of 0.01 mg/kg (grain) and 0.05 mg/kg (straw) according to the guidance documents SANCO/825/00, rev 8.1 and SANCO/3029/99/00, rev. 4. This submitted study has been validated in a proper manner.

Reference:	KCA 4.2
Report	Validation of Analytical Methods for the Determination of Flufenacet in Different Matrices of Plant Origin. Winter, O., Amann, S., 2021. Report S20-09167 (GLC-2019V). Eurofins Agroscience Services, Germany
Guideline(s):	Yes, SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Study Objective
The objective of the study was to validate an analytical method for the determination of flufenacet and its metabolites (flufenacet-oxalate (M1), flufenacet sulfonic acid sodium salt (M2), flufenacet-thioglycolate sulfoxide (M4) and FOE Cysteine (M23)) in wheat (grain and straw) according to the guidance documents SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1 of the European Commission, with an intended limit of quantification (LOQ) of 0.01 mg/kg in wheat (grain) and 0.05 mg/kg in wheat (straw). The LOQ of the flufenacet metabolites is defined as parent equivalent. They were fortified as a mixture in a

molar ratio 1/1/1/1. All analytes were determined as the common moiety 4-Fluoro-N-isopropylaniline (FOE 5043-aniline) and calculated and expressed as total residue of flufenacet.

After the experimental end of this study the new guideline SANTE/2020/12830, Rev.1, which supersedes guidelines SANCO/825/00, rev. 8.1 and SANCO/3029/99 rev. 4, was published. The data obtained within this validation study are mostly compatible with the new guideline.

Methods and Results

Analytical Procedure
In brief, samples of wheat (grain and straw) were extracted with water followed by oxidation with potassium permanganate and hydrolysis with sulphuric acid. Thereafter, the residues were purified by water steam distillation of the formed common moiety compound 4-Fluoro-N-isopropylaniline (FOE 5043-aniline).

Selectivity
Quantification was performed using LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.

Matrix Effects
Mean matrix effects were < ± 20 % for wheat (grain) and thus deemed to be insignificant, except for FOE 5043-aniline (fortified with flufenacet metabolites) at mass transition m/z 154→95 where the mean matrix effect was 23.7 %. However, the matrix effects at most individual concentration levels were > ± 20 % and deemed to be significant. Therefore, matrix-matched standards were used for quantification throughout the study.

Mean matrix effects were < ± 20 % for wheat (straw) and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of five (5) concentration levels ranging from 0.06 ng/mL to 4.0 ng/mL. This range corresponds to 0.003 mg/kg to 0.20 mg/kg for wheat (grain) and 0.015 mg/kg to 1.0 mg/kg for wheat (straw) and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract.

The calibration curves obtained for both mass transitions and all matrices were linear since coefficients of determination (R^2) were ≥ 0.98. Linear regression was performed with 1/x-weighting.

Quantification
Quantification was performed by using linear regression. The injections of the calibration solutions were spread evenly over the whole analytical sequence.

Accuracy and Precision
Accuracy was determined by fortification of control samples with known amounts of the test / reference items and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). The parent compound flufenacet was fortified separately from the metabolites. The flufenacet metabolites were fortified as a mixture in a molar ratio 1/1/1/1. All analytes were determined as the common moiety 4-Fluoro-N-isopropylaniline (FOE 5043-aniline) and calculated and expressed as total residue of flufenacet. The following recoveries were obtained:

Flufenacet (fortified with flufenacet)							
Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Mass Transition *m/z* 154→112 (Proposed for Quantification)							
Wheat (grain)	0.01	97, 94, 98, 102, 96	97	3.0	5	97	6.8
	0.1	100, 84, 89, 100, 107	96	9.7	5		
Wheat (straw)	0.05	92, 93, 93, 103, 87	94	6.2	5	91	6.1
	0.5	85, 88, 85, 88, 95	88	4.6	5		
Mass Transition *m/z* 154→95 (Proposed for Confirmation)							
Wheat (grain)	0.01	94, 93, 99, 98, 102	97	3.8	5	96	5.6
	0.1	96, 86, 90, 99, 103	95	7.2	5		
Wheat (straw)	0.05	90, 91, 92, 97, 90	92	3.2	5	89	4.4
	0.5	83, 86, 86, 87, 89	86	2.5	5		

Fortified with flufenacet, determined as FOE 5043-aniline and calculated as flufenacet.

Flufenacet (fortified with a mixture of metabolites)							
Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Mass Transition *m/z* 154→112 (Proposed for Quantification)							
Wheat (grain)	0.01	72, 68, 70, 73,78	72	5.2	5	73	4.3
	0.1	75, 71, 77,71, 72	73	3.7	5		
Wheat (straw)	0.05	73, 72, 71, 69, 70	71	2.2	5	71	2.1
	0.5	71, 70, 74, 71, 72	72	2.1	5		
Mass Transition *m/z* 154→95 (Proposed for Confirmation)							
Wheat (grain)	0.01	71, 71, 73, 70, 70	71	1.7	5	71	2.7
	0.1	75, 69, 72, 69, 70	71	3.6	5		
Wheat (straw)	0.05	73, 72, 71, 68, 70	71	2.7	5	71	1.9
	0.5	72, 70, 71, 71, 71	71	1.0	5		

Fortified with a mixture of flufenacet metabolites (flufenacet-oxalate (M1), flufenacet sulfonic acid sodium salt (M2), flufenacet-thioglycolate sulfoxide (M4), FOE Cysteine (M23) in a molar ratio 1/1/1/1, expressed as parent equivalents), determined as FOE 5043-aniline and calculated as total residue of flufenacet.

All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg (0.05 mg/kg and 0.5 mg/kg for straw) for two (2) mass transitions are within 70 – 110 % with relative standard deviations ≤ 20 % and thereby comply with the standard acceptance criteria of the guidance documents SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The LOQ is the lowest validated fortification level and was thus successfully established at 0.01 mg/kg in wheat (grain) and at 0.05 mg/kg in wheat (straw) for the two (2) mass transitions.
Stability of Analyte(s) in Stock and Fortification Solutions
All analytes were found to be stable for at least 91 days when prepared in methanol and stored at typically 1 °C to 9 °C in the dark.

Stability of Analyte(s) in Solvent Calibration Solutions
FOE 5043-aniline was found to be stable for 14 days when prepared in acetonitrile/water (1/9, v/v) + 0.1 mL/L acetic acid and stored at typically 1 °C to 9 °C in the dark.

Stability of Analyte(s) in Sample Extracts
FOE 5043-aniline was found to be stable in final extracts of wheat (grain) for at least 12 days and in final extracts of wheat (straw) for at least 10 days when stored at typically 1 °C to 9 °C in the dark.

Conclusion
The method was found to be valid according to the guidance documents SANCO/825/00, rev 8.1 and SANCO/3029/99/00, rev. 4 for the determination of flufenacet and its metabolites in wheat (grain and straw) with the tested LOQ of 0.01 mg/kg (grain) and 0.05 mg/kg (straw).